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Studies on Renal Function in Dogs.

Extraction values for PAH obtained by percutaneous catheterization and clearance studies on single kidneys.

By

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Well-proved methods for the study of renal function in human beings involve catheterization of the renal vein to determine extraction values for diadrast and PAH. Previous investigations on dogs have utilised either exposure of the renal vein or explantation of the kidney. The latter technique was developed by RHOADS (1934) and permits repeated clearance studies. Dogs prepared in this manner have been used for determination of extraction values for diodrast and PAH by WHITE (1940), CORCORAN, SMITH and PAGE (1941) and PHILLIPS *et al.* (1945). The question has been raised, however, whether or not the extraction values obtained by this means were affected by alterations in the position and blood supply of the kidney.

A technique for catheterising the renal veins of dogs has been developed by KREIENBERG (1954) from the method described by WARREN, BRANNON and MERRIL (1944) for human beings. KREIENBERG entered the jugular vein to introduce a ureteral catheter into the renal vein. The method used for the present work was that described by HELANDER, ÅSHEIM and ÖDMAN (1958) for percutaneous catheterization of the renal vein from the femoral vein.

Clearance studies on single kidneys require simultaneous collection of urine from each kidney. The only means of accomplishing this in dogs is by surgical means. Since it was planned to carry out repeated clearance studies over an extended period, it was apparent that the methods based upon acute isolation of a ureter and insertion of a catheter were not suitable for the present work.

A method for explantation of the ureteral orifice and the surrounding bladder wall to the abdominal wall has been described by DRAGSTEDT and DRAGSTEDT (1926). MC CAUGHAN and MANN (1931) modified the technique and explanted one ureter only. Urine was collected by inserting a catheter in the isolated ureter. A similar surgical procedure was used for the present studies but urine was collected by the means to be described below.

Methods.

A. Percutaneous selective catheterization of the renal vein.

A detailed description of the technique has been given in a previous paper (HELANDER, ÅSHEIM and ÖDMAN, 1958). A radio-opaque catheter with the tip bent in a half-circle is introduced into the femoral vein by SELDINGER's method and with the aid of fluoroscopy, it is placed in the renal vein.

B. The collection of separate urine samples from each kidney.

I. Surgical technique for transplantation of the ureteral orifice.

Mebumal®[1] given intravenously at 25 to 30 mg per kg body weight was used for anaesthesia. The orifice of the left ureter was transplanted to the ventral abdominal wall by the method of MC CAUGHAN and MANN. About 3 to 4 cm lateral to the linea alba and somewhat caudal to the navel appeared to be the most suitable site for the collection of urine and avoidance of skin lesions. About 10 cm of the distal portion of the ureter together with the ureteral orifice and the surrounding bladder wall were dissected free with the associated blood vessels and sutured to the abdominal wall. The ovarian vein of the same side was ligated to avoid contamination with extrarenal blood from this vessel when taking blood samples from the renal vein.

[1] The formula of Mebumal is as follows:

5-ethyl-5 (1-methylbutyl)-malonylcarbamide (Mebumal)	1.8 g
Mebumal sodium	4.0 g
Methane	25 g
Spir. conc.	15 g
Glycerin	12.5 g
Aq. steril.	100 ml

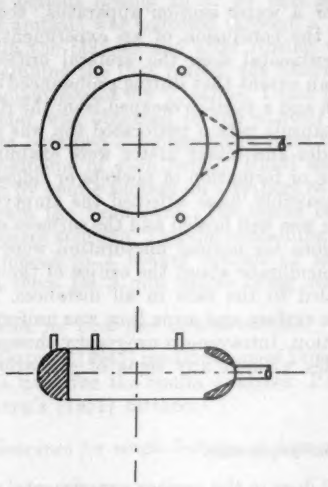

Fig. 1. Diagram of device for collecting urine from implanted ureters. Upper: View from above of ring with side tube. Lower: Side-view of ring with barb-like projections and side-tube.

II. Collection of urine.

Urine from the right kidney was collected by means of a Foley catheter placed in the urinary bladder, with the dogs in the supine position.

The following technique was used to collect urine from the left kidney. A ring, shown in Fig. 1, was constructed to have an outer diameter of 4.5 cm, an inner diameter of 4.0 cm, and a height of 7 mm. Five barb-like projections were placed at a uniform distance from one another about the border of the ring. A 1 cm long conical depression opening into a tube to which a polyethylene catheter could be attached was made in the smooth inner wall of the ring. The ring was sutured to the abdominal wall so that the ureteral orifice opened into its centre. Steel wire was used for a tobacco-pouch suture with a diameter of about 5.5 cm and was arranged so that the suture was exposed above the skin at points corresponding to the projections on the ring. When the suture was tightened by hooking the exposed portion of the suture over the projections on the ring the skin was drawn against the sides of the ring and formed a concavity with the orifice of the ureter in its centre. When tightening the suture, it is important to see that the skin about the ureteral orifice is not stretched and stasis produced. Urine flowed through the conical hole in the side of the ring and was collected in a

flask by means of a water suction apparatus, the ring and sutures were removed at the conclusion of an experiment.

On a few experimental dogs the ureteral orifice after healing was deviated to such an extent that during pronounced diuresis, a stream of urine gushed forth and a portion escaped from the ring. To correct this, a conical rubber capsule with a perforated top was applied to the ring.

When the bladder and intact ureter were examined at autopsy, no signs of induration or formation of pockets or ridges could be detected which could conceivably have affected the emptying of the bladder. The operation site was well healed and the surfaces smooth and regular. Possible obstructions for normal micturation were not observed. The bladder mucous membrane about the orifice of the transplanted ureter satisfactorily healed to the skin in all instances. The ureteral orifice projected over the surface and urine flow was uniform during the entire period of observation. Intravenous urography showed a normal appearance on both sides without any signs of dilatation of the ureter or renal pelves.

C. Experimental arrangements.

The urine of all dogs in the various experimental series was routinely examined for specific weight, protein, NPN, glucose, and sediment. Blood samples were examined for RBC and total WBC counts, Hb, haematocrit, NPN, and sedimentation rate. Only healthy animals with values within the normal range were used.

Before each experiment the dogs were fasted for 24 hours. Five hundred ml water were given by stomach tube 30 min before anaesthesia was induced. The animals were intubated and maintained under light Mebumal® anaesthesia. The depth of anaesthesia was continuously checked by means of the corneal and toe reflexes and additional small doses of Mebumal were given as required. The animals were placed on their backs during the entire experimental procedure which never extended beyond three hours. Rectal temperature was taken on several occasions without any significant deviation being noted. A polyethylene catheter of the usual type was placed in the femoral artery and a radio-opaque polyethylene catheter was introduced through the femoral vein into the renal vein. Physiological saline solution was administered continuously through the vein catheter at a rate of approximately 80 drops per min. The arterial catheter was connected to a kymograph for continuous recording of the mean blood pressure. On three instances, recording of blood pressure was commenced before anaesthesia was induced. There were no significant differences between the blood pressure values obtained before and during anaesthesia. After introduction of the catheter, initial doses of PAH and inulin were given, approximately 15 and 40 mg per kg body weight respectively. PAH and inulin were then given continuously by infusion through a catheter inserted in a leg vein. The plasma concentration of PAH was maintained at about 2 mg per cent and of inulin between 15 and 25 mg per cent. Forty minutes after administration of the initial dose and commencement of

infusion, clearance periods lasting 15 or 20 min were begun. Urine from non-prepared animals was collected through a Foley catheter by uniform pressure on the bladder and after rinsing with 0.9 % saline and air. Before beginning the first period, the bladder was repeatedly rinsed with large quantities of 0.9 % saline. Urine from animals with a transplanted ureter was obtained in the manner described earlier. Blood samples were obtained simultaneously from the femoral artery and vein at the midpoint of each period. Blood samples were taken from the renal vein by gentle suction on a syringe to avoid contamination with blood from the vena cava. The inside of the syringe was moistened with heparin solution. The vial containing the blood samples was immediately placed in a beaker containing ice water and was allowed to remain there for 5 min before being centrifuged.

D. Analytical methods for clearance determinations.

JOSEPHSON and GODIN's (1943) modification of CORCORAN and PAGE's (1939) method was used for the inulin analyses. PAH analyses were carried out by SMITH's (1951) method.[1]

Inulin and PAH clearance for single kidneys of dogs with a transplanted ureteral orifice.

Material. Ten bitches prepared in the manner described earlier and weighing between 18 and 25 kg were used for this experimental series. Renal-clearance studies were carried out on three of these animals before as well as after transplantation of the ureter to the abdominal wall.

Results.

The mean values for diuresis, inulin and PAH clearance and the extraction values of PAH are given in Table I for the three dogs which were investigated before and after operation. The post operation values include the mean values for the individual periods for each side and the mean function values for both sides together. It is apparent from the table that there were no significant differences in renal function between the various occasions.

Corresponding function values are given in Table II for the other seven dogs with a transplantated ureteral orifice. These values indicate that there is close agreement between the results obtained for both kidneys and that renal function varies within physiological limits during repeated experiments on one dog.

[1] All assays were done with a Beckman spectrophotometer, model B. Duplicate determinations were made of the inulin and PAH concentrations in each blood and urine sample, and the mean values were taken. In 50 consecutive duplicate tests of the inulin concentration, the coefficient of variation was 2.62 per cent; in consecutive duplicate determinations of PAH, the corresponding figure amounted to 1.45 per cent.

Table I.

Comparative renal function values before and after implantation of ureteric orifice, and values for right and left kidneys in operated dogs.

All figures are the mean values from the clearance periods.

Dog	Expt. no.	Urine ml/min		C_{In} ml/min		C_{PAH} ml/min		$\frac{C_{In}}{C_{PAH}}$		E % PAH		Combined mean values			
		R	L	R	L	R	L	R	L	R	L	Urine ml/min	C_{In} ml/min	C_{PAH} ml/min	E % PAH
F 13	31											1.9	45	127	76
F 13	48	1.2	1.0	27	27	176	174	0.15	0.16	83	81	2.2	54	350	
F 13	50	2.0	1.7	33	27	106	85	0.31	0.31	92	92	3.7	60	191	
F 17	23											2.2	83	303	71
F 17	34											4.3	97	167	83
F 17	43	1.3	1.3	46	42	122	93	0.38	0.45	91	—	2.6	88	215	91
F 17	44	1.0	1.3	47	53	128	151	0.37	0.35	82	—	2.3	100	279	82
F 20	36											5.1	86	270	87
F 20	47	2.0	2.1	35	46	144	162	0.24	0.28	93	90	4.1	81	306	93—90
F 20	49	2.0	2.0	38	39	95	102	0.40	0.37	82	75	3.7	77	197	82—75

Table II.

Comparison between inulin and PAH clearance for an additional seven animals.

Dog	Expt. no.	Urine ml/min		C_{In} ml/min		C_{PAH} ml/min		$\frac{C_{In}}{C_{PAH}}$		E % PAH		Combined mean values			
		R	L	R	L	R	L	R	L	R	L	Urine ml/min	C_{In} ml/min	C_{PAH} ml/min	E % PAH
F 22	52	3.0	2.1	39	28	145	109	0.27	0.26	82	81	5.1	67	254	82—81
F 22	57	0.7	0.4	33	27	110	88	0.30	0.30	87	76	1.1	60	198	87—76
F 22	62	1.5	1.2	30	25	139	100	0.22	0.25	84	—	2.7	55	239	84
F 22	64	1.1	0.5	45	35	101	87	0.45	0.40	—	—	1.6	90	188	—
F 25	61	0.4	0.3	34	29	114	106	0.30	0.27		76	0.7	63	220	76
F 25	63	0.8	1.0	35	31	95	126	0.37	0.25	—	—	1.7	66	221	—
F 32	76	0.1	0.1	36	34	93	91	0.38	0.38		81	0.2	70	184	81
F 40	96	0.6	0.7	40	31	139	106	0.29	0.30	77	—	1.3	71	245	77
F 36	85	0.4	0.3	39	36	107	106	0.36	0.34		74	0.7	75	211	74
F 42	84	0.7	0.4	28	32	137	121	0.21	0.27	75	80	1.1	60	258	75—80
F 50	99	0.6	0.5	41	43	159	155	0.28	0.28	83	84	1.1	84	314	83—84

Table III.

Mean values of inulin and PAH-clearance in non-operated dogs.

Dog	Expt.	Urine ml/min	In ml/min	PAH ml/min	$\frac{C_{In}}{PAH}$
F 14	30	1.7	47	101	0.47
F 19	35	1.0	123	383	0.32
F 21	46	2.0	69	175	0.39
F 21	51	3.0	60	174	0.34
F 23	67	0.25	59	190	0.31
F 39	68	2.3	74	187	0.40
F 41	79	1.1	111	207	0.51

Renal extraction of PAH.

Renal extraction of PAH has been calculated by the formula $E = \frac{A-V}{A} 100$ in which E = per cent extraction, A = PAH concentration in arterial plasma, and V = PAH concentration in renal vein plasma. The results have been considered for two main groups, *i. e.* extraction values of PAH for dogs without and with a transplanted ureteral orifice respectively.

The first group includes 9 dogs without a transplanted ureteral orifice on which 10 clearance tests have been made. Selective catheterization was performed on the right side only.

Extraction values for the 10 prepared dogs were determined by 14 clearance tests. Catheters were introduced into both renal veins 9 times, 3 times into the left side only, and twice into the right side only. Between one and six extraction values were obtained during each clearance test.

Results.

Only extraction values for PAH are included in Tables IV and V. The additional information obtained during the clearance tests is given in Tables I, II, and III.

The following results were obtained for the non-prepared dogs. Extraction values of PAH obtained for individual periods for clearance tests fluctuated within a range of ten per cent. In most instances, however, the differences were much less. The mean extraction values for the various clearance tests was 82 per cent with a range of 75 to 90 per cent.

Table IV.

Renal extraction of PAH (E). Unoperated dogs.

P = Arterial plasma concentration of PAH in mg/100 ml

Dog	Expt. and P	E % PAH	Mean E %	Dog	Expt. and P	E % PAH	Mean E % PAH
F 13	21 1.40—1.15	86 87	87	F 21	51 3.05—2.85	82	82
F 14	30 2.35—1.70	79 71	75	F 33	67 2.15—2.30	76 81	79
F 17	34 3.00—2.71	78 87	83	F 34	68 2.35—3.00	79 75	77
F 19	35 1.83—1.75	86 93	90	F 41	79 1.95—2.70	79 85	82
F 20	36 2.75—2.20	91 82	87			83	M 82
F 21	46	77 3.05	77			Range: 71—93	Range: 75—90

The results obtained with the prepared dogs showed that the differences between extraction values of PAH during separate clearance tests were of the same magnitude as those obtained for non-prepared animals. Similar results were obtained for the mean values and the extraction values for each clearance test.

Close agreement was obtained when the extraction values obtained simultaneously for the left and right sides were compared.

Discussion and Conclusions.

The effect of anaesthesia on renal function of dogs has been thoroughly investigated by CRAIG, VISSCHER and HOUCK (1945), CORCORAN and PAGE (1943), and GLAUSER and SELKURT (1952). Barbiturate derivatives were used in these experiments. It has been pointed out that the depth of anaesthesia greatly affects renal function and that under deep anaesthesia, renal circulation, glomerular filtration, and urine excretion are markedly depressed. During light anaesthesia, glomerular filtration is unchanged and RPF decreases somewhat if anaesthesia is protracted. Urine excretion is not affected to any great degree during light anaesthesia. It is generally stated that blood pressure during anaesthesia is unchanged or increases slightly. CORCORAN and PAGE found that

arterial blood pressure rose from 130 mm Hg to about 150 mm Hg. Similar observations were made during the present experiments; blood pressure recordings showed an unchanged or slightly increased pressure. The mean arterial vessel pressure, however, was somewhat higher, about 160 mm Hg.

Since the preliminary steps of introducing catheters into the various blood vessels required a certain length of time, the question arose concerning the effect of prolonged anaesthesia upon the values obtained. GLAUSER and SELKURT have shown that after anaesthesia extending over 3—5 hours, some values for function are altered to a degree which necessitates their being accepted with reservation. The time required for the experiments described in this paper was so short that there was scarcely any chance of renal function being depressed.

MALUF (1949) and OGDEN et GAUDINO (quoted from Smith (1951)) among others, have pointed out that circulatory reflexes in the kidney may be initiated from the ureter by, for example, stimulation arising from the introduction of a catheter. Because of this, urine was collected by the technique described in the section dealing with methods.

The surgical procedures performed on these dogs were designed to obtain an intact unit consisting of the kidney, ureter and bladder for one side as a means of checking that the kidney with a transplanted ureter functioned normally.

The result demonstrates that the kidneys have functioned in a fully comparable fashion without one or the other kidney dominating. The differences which were obtained between the functional values for the left and right sides varied for the different testing occasions. Consideration of the general sources of error for the method, the different means of collecting urine, and that the ureters and renal pelves do not contract simultaneously offers a satisfactory explanation for the differences. More reliable and satisfactory collection of urine is probably obtained from the prepared side since manual evacuation of the bladder for the non-prepared side is associated with a risk that small amounts of urine might remain in spite of the careful technique and repeated rinsings. This factor may assume significant proportions, especially when diuresis is relatively low.

Only a small difference is obtained when the values $\frac{C_{In}}{C_{PAH}}$ for the different periods for each side and for both sides for the

Renal extraction of PAH (E). Dogs with implanted ureter.

Table V.

Renal extraction of PAH (E). Dogs with implanted ureter.
P = Arterial plasma concentration of PAH in mg/100 ml.

Dog	Expt. and P	E % Right	Mean Right	E % Left	Mean Left	Dog	Expt. and P	E % Right	Mean Right	E % Left	Mean Left	Dog	Expt. and P	E % Right	Mean Right	E % Left	Mean Left
F 13	48	83	82	75	80	F 20	49	80	82	80	76	F 40	96	75	77		
	2.10—	81		75			2.80—	83		84			3.20—	77			
	1.75	78		78			2.20	83		70			2.50	78			
		78		81				82		74							
		83		86						71		F 42	84	73	75	80	80
		89		86									3.15—	76			
													2.50				
						F 22	52	90	84	88	84						
							2.00—	85		83							
F 13	50	93	87	93	89		2.55	78		80		F 43	92	80	79	80	82
	2.15—	95		91									2.25—	78		84	
	1.50	88		88		F 22	57	88	88	72			1.75				
		85		85			2.50—	86		80	76						
		87		90			2.90	90				F 50	99	85	84	81	83
		91		91									2.05—	83		85	
													1.60				
F 17	43			91	88	F 22	62			81	84			Range	M 83		M 82
	1.55—			88			1.80—			86							
	1.35			84			1.55										
														Range:	Range:	Range:	Range:
F 20	47	95	92	98	89	F 32	74	78	81					73—97	75—92	70—98	74—89
	2.00—	89		84			3.10—	82									
	1.60	89		89			2.20	83									
		91		83				82									
		97															
						F 36	85			75	74						
							3.55—			73							
							3.00										

same periods are compared. On the prepared animals for which clearance tests were made before and after the operation, there were no depression in glomerular filtration or renal plasma flow. It was hardly possible during these experiments to obtain basal conditions of the type which can be established for human beings. No premedication was given the animals to avoid affecting renal function. The results for glomerular filtration and renal blood flow obtained with inulin and PAH clearance agree with those given by HOUCK (1948) in his extensive statistical study of clearance tests in dogs.

When compared with methods previously used to obtain blood from the renal vein, the selective method as described here offers many advantages. The surgical interference is of small proportions and the position of the kidney is not altered. This cannot be avoided if transplantation of the kidney is required to obtain blood samples. In addition, such procedures are associated with the risk of scar formation and induration about the operation site and consequent disturbances in the intrarenal blood circulation. The method described by KREIENBERG for catheterization through the jugular vein necessitates ligation of the jugular and eliminates the possibilities for repeated catheterization. Several catheterizations on each animal have been carried out by the method described in this paper. No complications occurred and at subsequent autopsy, no lesions could be observed in the renal vein or its main branches.

It has already been pointed out in the description of the method that care was taken not to penetrate too far into the branches of the renal vein to avoid trauma of the vein and to ensure that a blood sample could be aspirated without the flexible wall of the vein occluding the aperture of the catheter during suction. Since the catheter was introduced into one of the larger branches of the renal vein to a point corresponding to the central part of the renal pelvis, there was little opportunity for contamination with unwanted blood. It is quite likely, therefore, that the values obtained adequately represent the extraction capacity of the kidney. On the non-prepared dogs, samples were obtained from the right renal vein only in order to avoid possible contamination of blood from the ovarian vein which generally opens into the renal vein on the left side.

Examination of the extraction values of PAH for different periods obtained for either the right or left side shows differences

between periods which could be as great as 10 per cent on various occasions. These differences were obtained in spite of precautions to ensure that the position of the catheter was unaltered before taking blood samples. The possible errors arising from the analytical methods are so small that they cannot afford an explanation of the differences. It is possible that these differences in the extraction values of PAH resulted from variations in the capacity of the kidneys to excrete the PAH administered. Similar changes are known to exist in the case of renal blood flow and glomerular filtration. Another possibility is that the PAH diffused from the erythrocytes to the plasma during the time required to collect the blood sample and remove the erythrocytes by centrifugation. PHILLIPS *et al.* (1945) have demonstrated that diffusion does actually occur and they have estimated it to be about 5 per cent during the 5 min required for the collection of a sample and its separation in a cold centrifuge. A cold centrifuge was not available during the present work with the result that the time required for chilling a blood sample and centrifuging it varied between 8 and 10 minutes. Under these circumstances it is impossible to decide whether or not diffusion of PAH from erythrocytes could explain the differences in the extraction values as the result of delay during the separation of the blood samples or whether there were variations in the extracting capacity of a kidney during the course of an experiment. The most satisfactory course is to compare the mean extraction values obtained on every occasion.

Such comparisons demonstrate that there were no differences in the mean values for PAH extraction between the prepared and the non-prepared dogs. Furthermore, no differences could be observed between the extraction values obtained from the left and right sides of the prepared dogs.

The values obtained in these experiments agree with the results recorded for dogs by CORCORAN, SMITH and PAGE (1941). They utilised transplanted kidneys in their studies and determined extraction values by means of diodrast clearance to obtain a mean of 0.84 with range between 0.79 and 0.96. A similar method was used by PHILLIPS *et al.* (1945) to determine PAH extraction. After correction for diffusion of PAH from erythrocytes to plasma, extraction values between 0.87 and 0.94 were obtained. The selective vein catheterization method used in the present work offers equally good possibilities for studies of venous blood in dogs as do similar methods in human beings. Values between 85

and 95 per cent have been obtained for human beings by this method. The values obtained during these studies on dogs had a mean of 82 per cent and a range from 70 to 98 per cent. It appears, therefore, that dogs have somewhat lower extraction values of PAH than human beings.

Summary.

Inulin and PAH clearances and extraction values for PAH have been used to study the function of individual kidneys in dogs. A technique is described for collecting urine without catheterizing the ureter. Percutaneous catheterization of the renal veins has been utilised to determine both unilateral and bilateral extraction values for PAH. No significant difference from normal values was produced by the operation of one ureter. The values were practically equal for both sides. Extraction of PAH was somewhat lower in dogs than in humans.

Much of this work was carried out during the time Professor B. Carlström was head of the department of medicine, Veterinärhögskolan. We should like to thank him for the generous support and helpful advice he has given us.

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From the Pharmacological Institute, University of Uppsala.

Failure of Carbonic Anhydrase Inhibition by Acetazolamide to Alter Tonicity of the Aqueous Humour.[1]

By

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The carbonic anhydrase inhibitor, acetazolamide N. N. R. lowers the intraocular pressure partially or wholly by inhibiting the production of aqueous humour. The inhibition of flow has been found by many authors to be accompanied by a change in the composition of the aqueous humour (for references, see review by BECKER 1957).

The most widely accepted theory (KINSEY 1950 a) concerning the formation of aqueous humour postulates that the osmotic pressure difference of about 100 mm Hg between the aqueous and the plasma is the principal force driving the flow of aqueous humour.

Therefore, it was of interest to see whether the changed composition of the aqueous following acetazolamide implies a reduction in the osmotic pressure gradient between aqueous humour and plasma.

Methods.

I. General Methods.

Animals: Albino rabbits were used throughout. They were kept in the cages of the laboratory for a minimum of one week before being subjected to the experimental procedures. The rabbits were of different

[1] The main results of the present investigation were communicated by E. BÁRÁNY at the 3rd Glaucoma Conference of the Josiah Macy, Jr. Foundation, January 8—10, 1958.

[2] Fellow of the Consiglio Nazionale delle Ricerche, Roma. Permanent address: Clinica Oculistica, Università, Naples.

sexes and were received from different distributors. They were fed oats, fresh hay and water *ad lib.*

Determination of intraocular pressure: This was done with a certified Schiøtz tonometer (tested according to the committee on standardization of tonometers, American Academy of Ophthalmology and Otolaryngology, Dr. E. J. BALLINTINE. It was found to compare favourably with the standard instrument also when tested on rabbit eyes) using the 5.5 g weight. The mean of two readings on every eye was expressed in mm Hg, using a calibration curve for eyes of rabbits under anaesthesia (WISTRAND 1958). A 2 % solution of lidocain (xylocain®), was applied topically to the cornea. Lidocain given in this way has no influence on the intraocular pressure.

Outflow pressure: This is the measured intraocular pressure in mm Hg minus the episcleral venous pressure (GOLDMANN 1954). The latter has been taken to be 9 mm Hg throughout (KORNBLUTH and LINNÉR 1955, AURICCHIO 1958).

Solutions: The sodium salt of acetazolamide N. N. R. (diamox®) and the control substance sulphadimidine (diazil®) were used as 1 % (w/v) solutions, which had been made isotonic to 0.9 % NaCl solution by adding solid NaCl. In the experiments with nephrectomized rabbits 3.3 % (w/v) solutions of diamox® and diazil® were used instead.

As a general anaesthetic allyl-isopropyl-barbituric acid, (numal®), was used.

Statistics: Mean values $\pm$ S. E. were calculated and compared by the "Student's" t-test. The statistical tables of FISHER and YATES (1953) were used.

II. Determination of the osmotic pressure gradient between anterior aqueous humour and plasma.

This was done by means of the Hill-Baldes thermoelectric vapour pressure method (BALDES 1934) as modified by BÁRÁNY (1947) and KINSEY (1950 b) and used by AURICCHIO (1958).

Microthermocouples of 20 Ω resistance containing 4 junctions in series were made from 0.05 mm diameter constantan and manganine wire. These were insulated by coating them with lucite. The loops of the couples were made wettable by painting them with a very dilute solution of celloidin in alcohol-ether and the portions between the loops were made water-repellent by painting with paraffin. This treatment, which was used by BÁRÁNY (1947) prevents the drops from either falling off the loops or creeping along the branches of the couple. The constant humidity chamber was the large model described by BALDES and JOHNSON (1939). It was immersed in a tank containing 60 litres of water and allowed to equilibrate at room temperature. Stirring and further thermal control were not necessary. The tank was surmounted by a tent of polyvinylchloride with a lucite window and slits for the hands of the operator. A binocular microscope ($\times$ 6) was mounted outside, and was focussed through the window for the loading and checking of the thermocouples. These were washed carefully after each contact with the solution and dried over silicagel for one hour before further use.

The thermovoltages generated were read on a Zernicke-C-galvanometer (Kipp, Delft, Holland) or recorded on an Elektronik strip chart instrument through a breaker-amplifier (mod. 14, Liston-Becker, Stamford, Conn.)

Calibration of the thermocouples for osmotic pressure differences was performed using primarily a glucose solution of 305 mM. The calibrating solutions were made by adding small amounts of distilled water to the same stock solution. In expressing the results, the osmotic activity coefficient of glucose was taken as 1.00.

Using standard solutions the standard error of the single determination with one thermocouple was $\pm$ 0.68 mOsm./kg (54 determinations) with the galvanometer and $\pm$ 0.40 mOsm./kg with the breaker amplifier (39 determinations).

The method error (from the differences between 3 thermocouples) using aqueous humour and plasma was $\pm$ 0.85 mOsm./kg (149 determinations) which means that the standard error of the method amounted to about 0.2—0.3 % of the osmolar concentration of plasma or aqueous.

In the following procedure every determination is the median value of three measurements with three different thermocouples except in the case of posterior aqueous, where only two couples were used. The measurements were made at room temperature (+ 21° C) and the humid chamber was filled with alveolar air before each measurement.

III. Technique for taking the samples of plasma and aqueous humour.

Anterior aqueous humour: About 0.1 ml was taken by means of a specially constructed syringe with an extremely small dead space (0.001 ml). The aqueous was kept anaerobically in the syringe until its osmotic pressure was determined.

Posterior aqueous humour: This was taken by means of an ordinary tuberculin syringe, the dead space of which was filled with mercury to prevent entrance of air. If the aqueous did not spontaneously enter the syringe it was cautiously aspirated. If necessary the needle was moved to another direction. The rabbit was discarded if changing the position of the needle twice gave no result. This happened in 8 of the 28 rabbits.

Plasma: In the anterior chamber experiments, heart blood was taken (about 3 ml) under paraffin oil about 3 min after the keratocentesis. The plasma was separated anaerobically within 5—10 min by centrifugation at room temperature. In the posterior chamber experiments, blood was taken 4—5 min before the chamber puncture. The plasma was kept anaerobically in a glass syringe before the determination of its osmotic pressure. The quality of the blood was observed because Meschia and Barron (1956) have found that in dogs the venous blood is more osmotically active (1.2 mOsm./kg) than arterial blood.

The blood was prevented from coagulating by adding heparin in three different ways: in one series of the experiments "wet" heparin (5 % heparin dissolved in 0.9 % NaCl) was drawn into the dead space of the syringe (volume about 2 % of the sample). In another series

1.0—1.2 mg of dry heparin was put at the bottom of the centrifuge tube. In the third series the 5 % heparin was given intravenously to the rabbits (0.2 ml/kg) 30 min before the heart puncture.

Control experiments were performed to see whether or not heparin added in the two "external" ways influenced the osmotic pressure of the plasma. Six ml of blood was taken from each of 6 heparinized rabbits and each blood sample separated into two parts, each containing 3 ml. One of these parts from each sample served as control and the other was further heparinized by the following procedures. In two instances, the blood was drawn up in the same syringe "wetted" with heparin as in the real experiments. In four instances, the blood was put into centrifuge tubes containing 1.2 mg of dry heparin. Thereafter all the 12 parts were handled in the same way. The difference in osmotic pressure between the parts heparinized *in vitro* and the control parts for every individual blood sample was determined. These differences (heparinized control) were: wet method, — 1.21, + 0.78, dry method, + 0.50, + 0.60, + 0.36, — 0.11 mOsm./kg. This means that in our experiments heparin had no significant influence upon the osmotic pressure of the plasma. This finding is confirmed by the results of the experiments where heparin was administred intravenously. The osmotic pressure gradient between plasma and aqueous humour in these experiments did not differ noticeably from those where heparin was added *in vitro*.

Experiments with the acetazolamide added to the blood in vitro.

Acetazolamide is taken up by the red corpuscle (MAREN *et al.* 1954 a) and therefore might conceivably influence the shift of ions between the corpuscles and the plasma due to inhibition of the carbonic anhydrase. This influence could change the osmotic properties of the plasma. Therefore, the following experiment was performed: 10 ml of heparinized heart blood was separated into 2 samples. To these two samples were added equimolecular amounts of acetazolamide and sulphadimidine respectively to give concentrations (50 μg/ml) which approximately corresponded to the concentrations of the two substances in the *in vivo* experiments. The two blood samples were allowed to stand anaerobically at room temperature for 5 hours before the plasmas were separated and the difference in osmotic pressure between them was measured. The result — 0.03 mOsm./kg (acetazolamide plasma — sulfadimidine plasma) shows that the uptake of acetazolamide by the red corpuscles does not change the osmotic properties of the plasma.

IV. Procedure:

Three different types of experiments were performed. In one series the gradient between the *posterior* aqueous and plasma was measured about one hour after the intravenous administration of acetazolamide.

In the other two series the gradient between the *anterior* aqueous

was measured under steady state conditions where the concentration of acetazolamide had been kept at a high level for a long period.

1. *Determination of the gradient between the posterior aqueous and the plasma.*

One series of ten rabbits received 10 mg/kg acetazolamide i.v. A second series of ten rabbits received 10 mg/kg sulphadimidine i.v. The animals were kept wrapped in bags in order to keep them still. 67—75 min after the injection posterior aqueous humour and blood samples were taken. This was done without general anaesthesia and only 2 % lidocain without adrenaline was given topically.

The time of taking the samples corresponded to that at which acetazolamide has a maximal effect upon the eye pressure.

2. *Determination of the gradient between the anterior aqueous and plasma in continuously infused rabbits.*

Because of the difficulty in taking the posterior aqueous, which is probably reflected in the rather high scatter of the values obtained (see Table I) it was desirable also to determine the gradient between the more easily available anterior aqueous and the plasma.

Despite inhibition of the flow of aqueous humour by acetazolamide, most of the aqueous humour in the anterior chamber is renewed after 6 hours. (If it is assumed that the flow is reduced to half, it is about 0.6 %/min, which gives 12 % "old" aqueous humour left in the anterior chamber after 6 hours.)

During continuous i.v. infusion of acetazolamide for 6 hours the eye pressure is kept reduced and the plasma concentration of acetazolamide is kept reasonably constant (WISTRAND 1958). The same technique for continuous infusion was used in these experiments.

Thirteen rabbits in each group were injected i.v. with 10 mg/kg of acetazolamide or sulphadimidine. Thirty minutes after this injection continuous infusion of test- and control substance was started. The infused dose was 0.2 mg/kg/min.

During the infusion the rabbits were tied in bags and received no food or water. After 6 hours the infusion was stopped and the

Table I.

Lack of influence of acetazolamide on osmotic gradient between posterior chamber aqueous and plasma.

Experimental animals received single intravenous injection of 10 mg/kg of acetazolamide, controls 10 mg/kg of sulphadimidine.

	Experimental	Controls
Number of rabbits	10	10
Mean body weight, kg	2.1	2.0
Time after injection, blood sample, min.	70.6	67.2
Time after injection, aqueous sample, min.	75.4	71.4
Excess osmotic pressure in aqueous, mOsm./kg water mean ± S. E.	6.8 ± 1.0	6.9 ± 1.3

rabbits were sedated with 0.4 ml/kg i.v. numal®. Thirty minutes after the injection of numal® the aqueous humour and blood samples were taken.

3. *Determination of the gradient between the anterior aqueous and the plasma in nephrectomized rabbits.*

Unlike sulphadimidine, acetazolamide has an effect upon the electrolyte composition of the plasma via the kidneys. In order to avoid these effects experiments with nephrectomized rabbits were performed.

Two groups of 19 and 20 rabbits each were nephrectomized (under numal® anaesthesia, 0.6 ml/kg) by ligatures around the pedicles of the kidneys. One group was injected intramuscularly with acetazolamide and one with sulphadimidine; the dose of each was 100 mg/kg.

The next day, after recovery in their cages with access to food and water, they were sedated with 0.4 ml/kg numal® intravenously. Thirty minutes after this injection tonometer readings were taken and the anterior aqueous humour and blood sampled. This was done on an average about 18 hours after the nephrectomy. At this time the concentration of acetazolamide in the plasma was determined in 4 of the rabbits. This was done using the technique of MAREN *et al.* (1954 b).

Results.

These are illustrated in Tables I—III.

The osmotic pressure gradient between aqueous humour and

Table II.

Lack of influence of acetazolamide on osmotic gradient between anterior chamber aqueous and plasma.

Experimental animals received single intravenous injection of 10 mg/kg acetazolamide followed by continuous infusion during 6 hours of 0.2 mg/kg/min. Controls received corresponding amount of sulphadimidine.

	Experimental	Controls
Number of rabbits	13	13
Mean body weight, kg	2.0	1.9
Number of blood samples which were arterial	7	8
Excess osmotic pressure in aqueous, mOsm./kg water mean ± S. E.	4.42 ± 0.63	4.28 ± 0.45

plasma was not significantly reduced by acetazolamide in the three experimental series.

The standard error of the method in a measurement of the osmotic pressure gradient between aqueous humour and plasma is about 3—4 times less than the standard error of the determination of the gradient, counting each rabbit as a unit. Thus, there is considerable variability in the gradient among the individuals. It is not clear which factors contribute to this variability, but part at least could be due to the procedure of taking the samples. Taking the aqueous strictly anaerobically does not seem to be very important, because BÁRÁNY (1947) reports almost the same standard error using a similar technique but without the anaerobic precautions used in our experiments. The reason for the greater standard error of the determination of the gradient in the first series is also not clear. This could depend upon the difficulties in taking the posterior aqueous and also upon the fact that these animals were not sedated when taking the samples. The gradient between the posterior aqueous and plasma appears at first glance to be higher than the gradient between the anterior aqueous and plasma. However, the figures are not comparable, since the rabbits in the three experimental series were handled in different ways.

KINSEY (1953) found no difference in osmotic activity between posterior and anterior aqueous.

The intraocular pressure of the nephrectomized control rabbits that received sulphadimidine was on an average lower than that (21.6 mm Hg) found in normal rabbits, measured with the same tonometer (WISTRAND 1958). This lower pressure could be due to the general anaesthesia or to the nephrectomy. Nevertheless,

Table III.

Effect of acetazolamide on intraocular pressure despite lack of effect on osmotic pressure gradient between anterior chamber aqueous and plasma.

Both groups of animals were nephrectomized. Experimental animals were given 100 mg/kg acetazolamide i.m. immediately after nephrectomy, controls received the same amount of sulphadimidine.

	Experimental	Control
Number of rabbits	20	19
Mean body weight	1.9	1.9
Interval between injection and tonometry, hours	18.2	18.5
Intraocular pressure[1], mm Hg mean ± S. E.	14.13 ± 0.78	18.70 ± 0.98
Number of blood samples which were arterial	16	16
Excess osmotic pressure in aqueous, mOsm./kg water mean ± S. E.	3.83 ± 0.24	3.88 ± 0.40

[1] Not measured in 2 control and 1 exptl animals.

acetazolamide significantly ($p < 0.01$) reduced the eye pressure and compared with the sulphadimidine animals this lowering was 37 % of the outflow pressure, the effect on which is about the same as that of the same dose of acetazolamide in normal non sedated rabbits (WISTRAND 1958). The plasma concentration of acetazolamide in the four experiments was 6.7, 38.5, 17.0 and 25.8 µg/ml. This shows that acetazolamide was still present in the plasma 18 hours after the administration and in a concentration which is effective on the intraocular pressure (WISTRAND 1958).

No correlation between the gradient and the intraocular pressure for each individual rabbit could be seen.

Discussion.

ROEPKE and HETHERINGTON (1940) and KINSEY (1951) have measured the osmotic pressure gradient between aqueous humour and plasma in the rabbit using the thermoelectric method. Our values for this gradient are of about the same magnitude as found by KINSEY (equivalent 3.0 mM NaCl/kg water) in unanaesthetized rabbits, and higher than that reported by ROEPKE and HETHERINGTON (equivalent 1.9 mM NaCl/kg water) in anaesthetized animals. LEVENE (1958) found the gradient to be 2.0 mOsm./kg water by the cryoscopic method in unanaesthetized rabbits.

Of 85 determinations of the gradient in our series (aqueous-plasma) only 2 were negative. The mean values and the standard

errors show that certainly aqueous humour is osmotically more active than plasma of arterial or venous heart blood.

The gradient in the nephrectomized rabbits was not lowered significantly and they responded normally to acetazolamide. LANGHAM and LEE (1957) report a lowering of the eye and blood pressure and an increased permeability of the blood-aqueous barrier after nephrectomy. That the effect of nephrectomy upon the blood-aqueous barrier can not be very strong is shown by the fact that the gradient is almost normal in these animals. LINNÉR and FRIEDENWALD (1957) also report that nephrectomy did not change the fluorescein appearance time in the rabbit. This indicates that the flow of aqueous was not influenced. BECKER (1955) showed that acetazolamide lengthened the fluorescein appearance time and hence reduced the flow of aqueous in nephrectomized rabbits.

Acetazolamide, 10 mg/kg i.v., to rabbits with intact kidneys lowered the intraocular pressure with at least 2 scale divisions (tonometer reading Schiøtz, 5.5 g weight) in about 70 % of the animals. This corresponds to a lowering of the outflow pressure and hence the rate of secretion by about 30—40 % (WISTRAND 1958). The nephrectomized rabbits in these experiments responded to acetazolamide with a similar drop in outflow pressure in spite of a lower initial pressure. This means that the flow of aqueous must have been inhibited in almost all of our acetazolamide rabbits.

One theory about the effect of acetazolamide is that it exerts its effect on the flow of aqueous by inhibiting the secretion of a special solute or electrolyte into the posterior aqueous. This might lower the osmotic activity of the posterior aqueous and thus the flow of water which is thought to be osmotically drawn into the posterior aqueous by the secreted electrolytes.

Furthermore it seems reasonable to assume that a certain lowering of the osmotic activity of the aqueous would be accompanied by a roughly proportional inhibition of the aqueous flow, if it is true that this osmotic force is the dominant one.

However, no reduction in the gradient was found, which is in agreement with the work of LEVENE (1958). Before accepting our result with its implication for the theories of aqueous humour formation it is necessary to discuss possible sources of error in the experiments.

According to the osmotic theory, water enters mainly through the posterior chamber. The rate of flow of water should be approximately proportional to the actual osmotic pressure gradient between the posterior aqueous humour and plasma. In the normal animal under steady state conditions the posterior and anterior aqueous are probably isotonic (KINSEY 1951). For the moment, let it be assumed that this is the case also following acetazolamide. However, under non-steady state conditions it is by no means certain that anterior and posterior aqueous are isotonic, and comparison of the osmotic activities of the anterior aqueous and plasma under transient conditions might not give the actually driving osmotic pressure gradient.

Since the experiments with continuous infusions may not wholly represent steady state conditions, for instance due to the diuresis brought about by the acetazolamide, the experiments with nephrectomized animals were run. The agreement between the results of the two series indicates that lack of steady state conditions can not have been an important source of error.

The question remains if the virtual isotonicity between anterior and posterior aqueous is present also after acetazolamide, when flow is reduced. It is possible that metabolites from the tissues accumulate in the anterior chamber disproportionally when flow is slowed down. However, the figures of BECKER and CONSTANT (1956) show that there was no accumulation of lactate in the rabbit anterior chamber compared with the posterior chamber six hours after acetazolamide.

Moreover, in our posterior chamber experiments, we found the same lack of effect of acetazolamide on the gradient as in the anterior chamber experiments. We therefore feel justified in stating that acetazolamide affects water transport into the aqueous not indirectly over the osmotic gradient aqueous-plasma but in some other way.

In this connection it should be mentioned that ligation of one carotid artery in rabbits reduces the aqueous flow to roughly the same degree as does acetazolamide (KORNBLUTH and LINNÉR 1955) but does not change the osmotic activity of the aqueous humour of the ligated side (BÁRÁNY 1947).

Summary.

The carbonic anhydrase inhibitor acetazolamide affects the production of aqueous humour in rabbits without changing the steady state osmotic pressure gradient between aqueous and plasma.

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Species Differences in the Taste Qualities Mediated through the Glossopharyngeal Nerve.

By

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That the glossopharyngeal nerve carries fibres mediating taste and touch from the posterior third of the tongue was shown already in the middle of the 19th century (GUZOT and CAZALIS 1831, MORGANTI 1846, quoted from LEWIS and DANDY 1930). Electrophysiological studies on the glossopharyngeal nerve of the cat were made by ZOTTERMAN in 1935 and by PFAFFMANN 1941. It seems, however, as if most of the experimental work on taste reception has been carried out on the chorda tympani. A comparative study of the modalities mediated through the glossopharyngeal nerve is thus still lacking. Such a comparative analysis in monkey, cat, dog, rabbit and rat will be presented in this study.

Methods.

One Rhesus monkey, 7 cats, 10 dogs, 6 rabbits and 11 rats were used in this investigation. The animals were anaesthetized by intravenous or intraperitoneal injection of nembutal.

In order to expose the tongue the right mandible was resected. The glossopharyngeal nerve was reached after cutting and removing a part of the great cornua of the hyoid bone. The action potentials

were led off and the test solutions applied in the way previously described by COHEN, HAGIWARA and ZOTTERMAN 1954 and by LILJESTRAND and ZOTTERMAN 1956. The following solutions were used as stimulants: distilled water, 0.5 and 1 M sodium chloride, 0.02 M quinine chloride in Ringer's solution, 0.1 M acetic acid in Ringer's solution, 15 % saccharose, glycerine and saccharine in Ringer's solution.

Results.

As is well known the caudal part of the tongue is innervated by the glossopharyngeal nerve. In this series of experiments the tactile receptive field of the glossopharyngeal nerve was found to extend from the epiglottis to a borderline a few millimeters anterior to the circumvallate papillae. The area was strictly ipsilateral. In all species investigated (monkey, cat, dog, rabbit and rat) a tactile response was obtained from this area. The row of large fungiform papillae at the border of the caudal part of the tongue were found to be extremely sensitive to touch. There was a certain overlap between the receptive fields of the glossopharyngeal and the lingual nerves. This overlap was, however, restricted to a few millimeters (3 to 5 mm in the cat).

The afferent inflow of taste impulses in the unsplit glossopharyngeal nerve of the above mentioned species was recorded. The records of Fig. 1 were obtained from the monkey. In this species all the taste solutions used including distilled water caused an increased impulse discharge in the glossopharyngeal nerve when applied to the caudal part of the tongue. The impulse discharge is recorded directly by the lower beam of Fig. 1. The second beam gives an integration of the impulse activity. The upper tracing signals the moment of application of the taste solutions.

Record A is a control with Ringer's solution showing that in this case no mechanoreceptive fibres were stimulated by the flow from the burette on to the tongue. Distilled water, on the other hand produced a clearcut response shown as an upward deflection of the integrator beam (record B). Sodium chloride and quinine gave massive discharges in the nerve (C, D) and also acetic acid evoked a pronounced increase in the impulse activity (E). Records F, G and H finally present the effects of sweet-tasting solutions as saccharose, glycerine and saccharine applied to the tongue.

The cat behaved in a somewhat different manner. As shown in

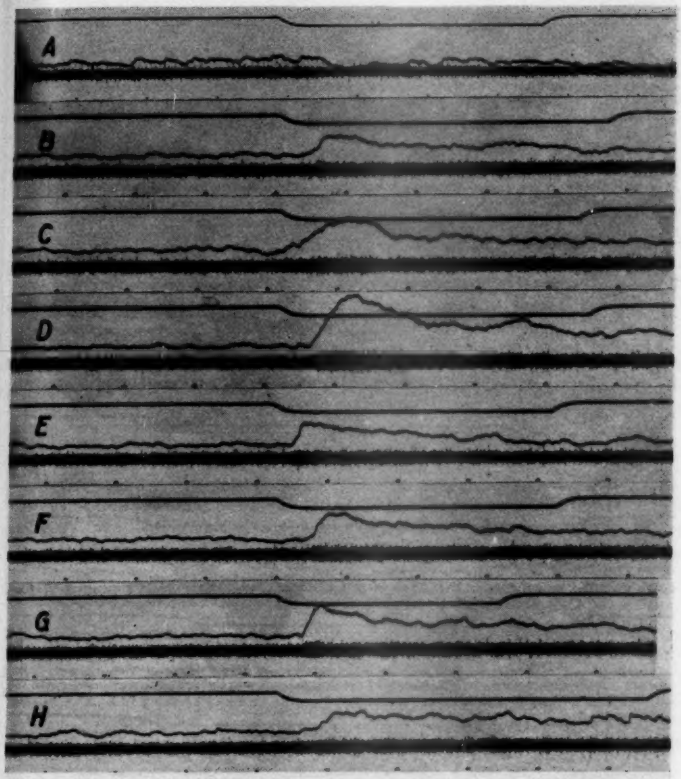

Fig. 1. Response of monkey's glossopharyngeal nerve to various solutions flowed over the tongue. A, Ringer solution; B, distilled water; C, 1 M NaCl; D, 0.02 M quinine hydrochloride in Ringer; E, 0.2 M HAc in Ringer; F, 15 % saccharose in Ringer; G, 15 % glycerine in Ringer; H, 15 % saccharine in Ringer. Time in seconds.

Fig. 2 it responded to salt (record C), bitter (record D) and acid (record E) as did the monkey but sweet-tasting solutions elicited no change in the level of spontaneous activity (F, G and H). It was also impossible to obtain any response to distilled water in the cat's glossopharyngeal nerve (B).

The other species investigated responded to salt, bitter and acid in a way similar to that described for the monkey and the cat. They also responded to saccharose and glycerine as was the case

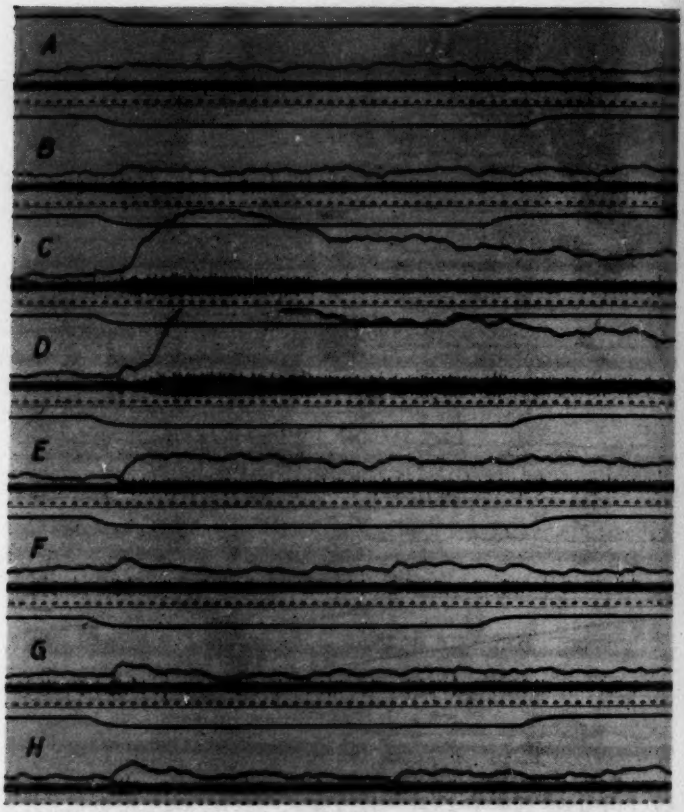

Fig. 2. Response of cat's glossopharyngeal nerve to various solutions flowed over the tongue. A, Ringer solution; B, distilled water; C, 1 M NaCl; D, 0.02 M quinine hydrochloride in Ringer; E, 0.2 M HAc in Ringer; F, 15 % saccharose in Ringer; G, 15 % glycerine in Ringer; H, 15 % saccharine in Ringer. Time 0.1 sec.

with the monkey. In this respect they thus differ from the cat. It should also be noted that in no animal but the monkey did saccharine or distilled water produce any increase of the activity in the glossopharyngeal nerve.

Response to warm and cold were seen in all the animals investigated except in the rat where warm solutions only produced a decrease in the spontaneous activity. Fig. 3 gives typical records

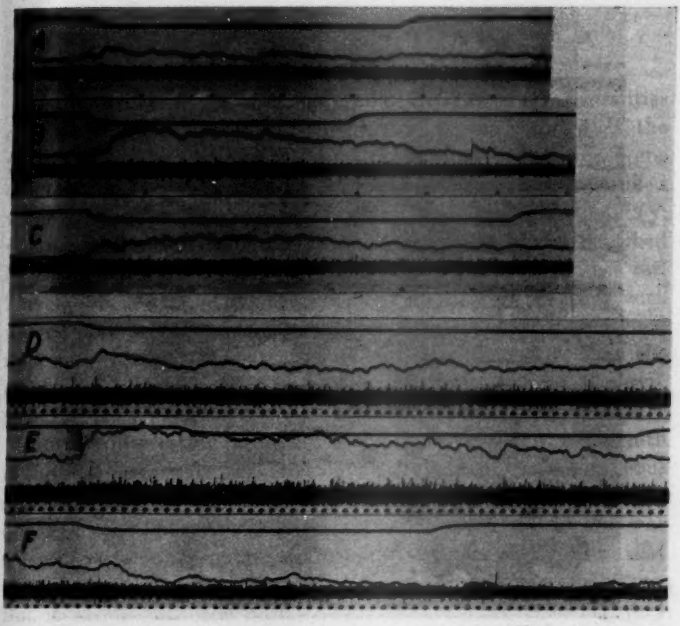

Fig. 3. Response of the glossopharyngeal nerve of monkey (A, B, C) and rat (D, E, F) to cooling and warming of the tongue. A, distilled water 32°; B, distilled water 20°; C, distilled water 42°; D, Ringer solution 30°; E, Ringer solution 25°; F, Ringer solution 45°. Time in A—C 1 sec, in D—E 0.1 sec. In E the moment of application is indicated by an arrow.

of warm and cold effects in monkey (A, B, C) and rat (D, E, F). Note that before each of the records D, E and F in Fig. 3 the tongue of the rat was allowed to adapt to room temperature. Solutions with temperatures ranging from 27° to 32° caused no change in the activity of the nerve. Temperatures from 32° to 50° all caused a decrease of the nervous discharge, while temperatures below 27° elicited clear-cut increase of the activity.

Table I gives a summary of the results now described. As a comparison also the results from earlier investigations on the lingual nerve or chorda tympani are included.

An interesting effect which was observed in all species investigated but was most clearly seen in the cat is illustrated in Fig. 4. A slight touch with a glass-rod on a circumvallate papilla gave a short burst of touch spikes recorded by the integrator as an upward

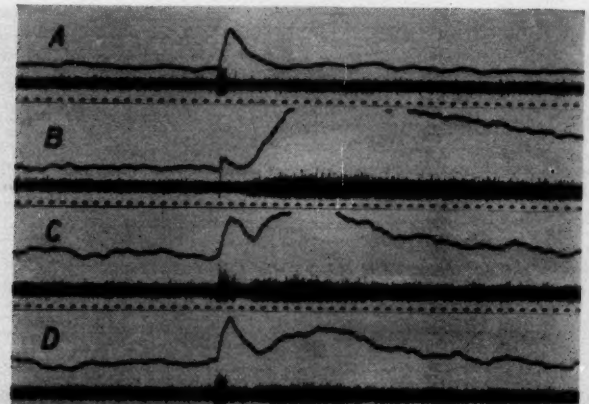

Fig. 4. Response of the cat's glossopharyngeal nerve to touch on an ipsilateral circumvallate papilla, before (A) and after (C—F) application of 0.02 M quinine hydrochloride solution to the tongue (B). Time 0.1 sec.

deflection of rather short duration (Fig. 4 A). After a taste solution had been squirted over the tongue touch similarly applied resulted in the same short burst of impulses. This initial burst was, however, followed by a rather longlasting discharge of smaller spikes appearing with a latency considerably longer than that of the tactile response (Fig. 4 C). The integrator shows a second deflection rising on the falling phase of the initial one. Fig. 4 B shows the application of the taste solution. Note that the moment of application is reflected by the integrator as a small hump, due to activity in touch fibres, and that the taste response which follows has about the same latency as the late discharge in record C. Records D, E and F were obtained after repeated washing of the tongue with Ringer's solution. It is seen that the second phase of the diphasic response to touch diminished in amplitude and finally disappeared (F).

Discussion.

The comparison presented in Table I between the modalities of the glossopharyngeal and the lingual nerves shows that the two nerves convey mainly the same types of afferent fibres. Some differences were, however, found. Thus a response to distilled water in the glossopharyngeal nerve was found only in the monkey, whereas water taste mediated via the chorda tympani was described by ZOTTERMAN (1956) in all species investigated except in the rat. This may of course be due to the technique used in this study with whole-nerve preparations. The fact that the monkey gives a good response with the same technique indicates, however, that the number of water fibres, if present in the glossopharyngeal nerve, must be rather small.

Another interesting difference is the lack of response to warmth in the rat. Warm solutions gave only a decrease of the spontaneous activity. This was probably due to a reduction of the cold fibre discharge particularly well seen in the rat when the tongue was exposed to room temperature. This does not necessarily mean that the rat totally lacks "warm" fibres in its glossopharyngeal nerve. But it shows that the number of "warm" fibres is either very low compared to that of "cold" fibres or that the glossopharyngeal "warm" fibres are of smaller dimensions.

An interesting problem is the lack of response to sweet in the cat. This was previously described by ZOTTERMAN (1935) and by PFAFFMANN (1941) for the glossopharyngeal as well as for the lingual nerve. The results now obtained are in agreement with these early findings.

On the contrary the monkey seems to be highly sensitive to all sweet-tasting substances. In this animal sweet-mediating fibres are found both in the chorda tympani (cf. ZOTTERMAN 1958, in press) and in the glossopharyngeal nerve. In the above mentioned investigation ZOTTERMAN also showed that the same single fibre in the monkey's chorda tympani responded to saccharose, glycerine and saccharine. This indicates that saccharine in the monkey acts as a sweet-tasting substance.

The most probable explanation of the double-humped response to touch of the circumvallate papillae after previous application of a taste solution illustrated in Fig. 4 is that the late discharge is due to firing of taste fibres. It is a well known anatomical fact that

Table I.

	Monkey		Cat		Dog		Rabbit		Rat	
	N. IX	Ch. tymp.[1]	N. IX	Ch. tymp.[2]	N. IX	Ch. tymp.[3]	N. IX	Ch. tymp.[4]	N. IX	Ch. tymp.[5]
Ringer	—	—	—	—	—	—	—	—	—	—
H_2O	+	+	—	+	—	+	—	+	—	—
NaCl	+	+	+	+	+	+	+	+	+	+
Quinine	+	+	+	+	+	+	+	+	+	+
Acetic acid .	+	+	+	+	+	+	+	+	+	+
Saccharose .	+	+	—	—	+	+	+	+	+	+
Glycerine ..	+	+	—	—	+	+	+		+	+
Saccharine .	+	+	—		—	—	—		—	—
Warm	+		+	+	+		+		—	
Cold	+		+	+	+		+		+	
Touch	+	+	+	+	+	+	+	+	+	+

[1] ZOTTERMAN 1958, in press.
[2] ZOTTERMAN 1935, HENSEL, ZOTTERMAN 1951, COHEN, HAGIWARA, ZOTTERMAN 1955.
[3] ANDERSSON, LANDGREN, OLSSON, ZOTTERMAN 1950, ZOTTERMAN 1956.
[4] ZOTTERMAN 1956.
[5] FISHMAN 1957, ZOTTERMAN 1956.

the taste buds of the papillae vallatae are situated rather deeply in the moat surrounding each papilla. It is therefore possible that a touch on the papilla opens the moat and thereby brings the taste solutions in contact with the taste buds. The view that the late discharge is a taste response is also supported by the fact that it is built up by impulses of a lower amplitude than that of the touch spikes forming the initial burst. A mechanical deformation of the papillae thus supports the effect of a tasting substance and probably improves the perception of taste.

Summary.

1. Taste solutions, distilled water, touch and thermal stimuli were applied to the tongue of monkey, cat, dog, rabbit and rat, and the effects on the impulse discharge in the glossopharyngeal nerve were recorded.

2. Salt-, bitter- and acid taste was found in all the animals investigated.

3. Response to sweet-tasting solutions was obtained in all these animals except the cat. Only the monkey did, however, respond to saccharine.

4. Water taste was found only in the monkey.

5. Effects to warm and cold were seen in all the animals investigated except in the rat where warm solutions gave no response.

6. A tactile response was found in all species.

7. The findings were compared with those previously obtained from the chorda tympani (Table I).

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Cardiovascular Effects of Direct Stimulation of the Carotid Sinus Nerve in Man.

By

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The reflex baro- and chemoreceptor control of the cardiovascular system has for many years aroused considerable interest, especially since it has recently been shown that the activity of the carotid baroreceptors is not only dependent on the distension of the sinus region by the blood pressure. The activity of at least one type of the receptors is also markedly affected by local changes in intramural tension, caused by the smooth muscle cells. Thus, local applications of vasoconstrictor drugs induce a considerable discharge of this baroreceptor type, and there is some experimental evidence, indicating that stimulation of sympathetic constrictor fibres to the carotid sinus wall can result in a similar receptor activation (for lit. see HEYMANS and NEIL 1958, pp 72—82). — Practically all our knowledge is derived from experiments on animals, however, and in man the carotid sinus mechanism has been investigated mainly by way of studies, where the baroreceptors have been activated by applying external pressure to the carotid sinus region.

The carotid sinus region is, however, routinely explored in surgical treatment of cancer in the neck. The unavoidable traction and manipulation of vessels and nerve trunks during such operations often provoke dramatic reflex cardiovascular effects, which

can at least partly be eliminated by local anaesthesia block of the afferent fibres, *e. g.* those in the vagal nerve (CARLSTEN *et al.* 1957, HAMBERGER *et al.* 1957). In five cases, where the cancer process made a careful dissection of the carotid sinus region necessary, the effects of direct stimulations of the exposed carotid sinus nerve have been studied. The present results have previously been briefly communicated (CARLSTEN *et al.* 1956).

Method.

All the five subjects studied underwent before the operations a careful cardiovascular analysis to exclude cases with any sign of heart disease. The experimental procedures were generally completed within 20 minutes with no side-effects whatsoever.

A complete exploration of the carotid sinus area was routinely performed, and it was then generally easy to identify and isolate at least the majority of the nerve fibres of the carotid bifurcation for electric stimulation.

As anaesthesia a combination of ether, nitrous oxide and Evipan was used. From the start of the operation the blood pressure, the pulse rate and the pulse amplitude were continuously recorded from an indwelling catheter, introduced percutaneously into the left brachial artery (BERNÉUS, CARLSTEN and HOLMGREN 1954). The catheter was connected to a strain-gauge manometer (Elema) operating a six channel electrocardiograph (Mingograph, Elema), where an almost linear correlation between pressure load and amplitude is obtained. For a control of the reflex nervous influences on the heart the electrocardiogram was also recorded on the Mingograph. — To allow measurements of the forearm blood flow, a waterfilled venous occlusion plethysmograph of conventional type was used. During actual flow recordings the hand circulation was temporarily obstructed by a cuff placed distally to the plethysmograph. A collecting pressure of about 30—40 mm Hg was for 6—10 seconds applied in a cuff, placed proximally to the apparatus, and the increases of forearm volume were registered by an ink writing piston recorder on a kymograph. — The respiratory movements were also recorded by way of a piezoelectric crystal, tied to a girdle around the thorax and in connection with one of the Mingograph channels. Before, during and after the sinus nerve stimulations, the blood pressure, pulse rate, electro-

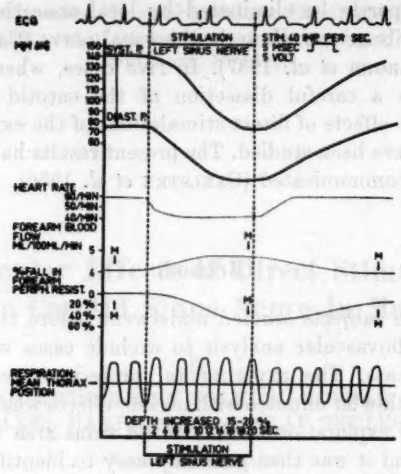

Fig. 1. A diagram illustrating the effect of stimulation of the left sinus nerve in man. Note the ectopic pacemaker of the heart at stimulation.

cardiogram and respiratory movements were continuously recorded, and repeated measurements of the forearm blood flow were performed. For stimulations a bipolar silver electrode was applied and rectangular stimuli were delivered from a Grass stimulator (model S4C). Generally the pulse duration was kept at 5 msec and the stimulation strength at 5—7 volts, which for a given frequency seemed to elicit a roughly maximal response, indicating that practically all the nerve fibres on the electrode were excited. The frequency was varied between 10 and 100 impulses per sec in some cases and each stimulation period lasted 10 to 20 sec.

Results.

A typical reflex response to electric stimulation of the carotid sinus nerve is shown in Fig. 1. As is seen from Fig. 1 a prompt fall in mean blood pressure, in pulse amplitude and in heart rate is induced by stimulation of the sinus nerve, together with a slight increase in forearm blood flow, implying a considerable fall

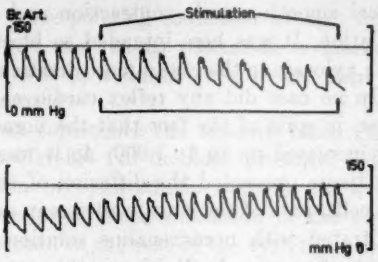

Fig. 2. Blood pressure response of sinus nerve stimulation during 10 seconds. Note decrease in pulse amplitude and also the rapid restitution after cessation of the stimulation.

in regional resistance to flow. The electrocardiogram shows an immediate shift to an ectopic pacemaker of a coronary sinus type. This arrhythmia belongs to the third group of coronary sinus rhythm as defined by SCHERF and GÜRBÜRZER (1958). The respiratory rate was only slightly changed, while the tidal volume seemed to increase somewhat during the stimulation, presumably an effect of chemoreceptor fibre stimulation. On cessation of stimulation, restitution started almost immediately and all reflex changes had in most cases vanished after 10—15 sec (see Fig. 2). — Similar effects were obtained in all cases, though the extent of the reflex changes varied somewhat, probably due to the fact that sometimes only a fraction of the baroreceptor fibres were in contact with the electrode.

Fig. 1 shows the effect of sinus nerve stimulation with 40 impulses per sec, but stimulations with 10 and 20 impulses per sec were also performed. The reflex response to 10 impulses per sec was considerably weaker but the effect obtained by 20 impulses per sec was almost as marked as that illustrated in the figure. The correlation between stimulation rate and magnitude of response varied to some extent in the experiments but maximal reflex responses were generally obtained by frequencies around 40—60 impulses per sec.

Since it is known from animal experiments that changes in intramural tension in the sinus region can profoundly increase the activity of at least one group of the baroreceptors, the exposed sinus region was in two cases painted with a noradrenaline solution

to induce a local smooth muscle contraction and hence a baroreceptor stimulation. It was here intended to block the exposed sinus nerve with xylocain in the case a more drastic reflex response should occur. In no case did any reflex cardiovascular response appear, however, in spite of the fact that the noradrenaline concentration was increased up to 1 : 1,000. As it was possible that the adventitial tissue prevented the diffusion of the drug to the smooth muscle cells, part of the sinus wall was in one of the cases cautiously infiltrated with noradrenaline solution by way of a syringe with a very fine cannula. Neither in this case did any reflex response occur except for a slight blood pressure fall during the short time while the manipulations were performed.

To see whether in man there is evidence of a sympathetic control of the smooth muscle cells of the sinus wall (see HEYMANS and NEIL 1958, pp 81—82), under which circumstances the increased intramural tension might excite the baroreceptors, the cranial sympathetic trunk was stimulated in a group of other cases (see FOLKOW and HAMBERGER 1956). The stimulation strength and frequency were here increased until a maximal pupillary response was obtained, but in none of these cases did any reflex fall in blood pressure or any reflex bradycardia occur.

Discussion.

The present study of the reflex cardiovascular effects of graded, direct stimulations of the carotid sinus nerve in man has given results, which are identical with findings in animal experiments (see HEYMANS and NEIL 1958 pp 56—67).

The prompt and often marked decrease of pulse amplitude calls for some remarks. The diminished pulse amplitude was in all probability caused by a fall in stroke volume, as the heart rate decreased simultaneously and there was evidence that the peripheral resistance was reduced. As the vagal fibres in man do not seem to exert any negative inotropic effect on the ventricles (CARLSTEN *et al.* 1957), the reduced stroke volume must then be a consequence of the generalized reflex inhibition of sympathetic tone. This will not only eliminate the positive inotropic effect on the myocardium, exerted by the accelerans fibres, but will also cause a rapid relaxation of the capacity section of the vascular bed. This in its turn means that a certain pooling of blood takes

place with a corresponding reduction of the venous return to the heart. When the venous relaxation is marked enough, the stroke volume will be reduced even if the heart rate is simultaneously decreased.

The fact that sometimes the forearm blood flow increases in spite of a considerable fall in blood pressure, indicates that the reflex inhibition of sympathetic tone on an average causes a somewhat more pronounced dilatation of the resistance vessels in the muscles than in the rest of the systemic vascular bed. This is often seen also in animal experiments when a pure inhibition of sympathetic tone is induced, and should not be taken as any evidence of *e. g.* activation of specific dilator fibres. It is known from animal experiments that the sympathetic and parasympathetic dilator fibres are not activated on stimulation of baroreceptor fibres (FOLKOW 1955).

The utilized stimulation strength seemed to be intense enough to allow an excitation of practically all the nerve fibres from the carotid sinus, that were in actual contact with the electrode. When under such circumstances the frequency was stepwise increased, a relatively marked reflex response was seen already at 10 impulses per sec, while maximal responses were obtained at a frequency range of 40—60 impulses per sec, which is in fair agreement with findings in similar studies in animals (DOUGLAS and SCHAUMANN 1956). — If opportunity is given, stimulations at a constant rate but with increasing voltage will be performed to determine whether also in man the sinus nerve contains fibre groups with definite differences in stimulation threshold and consequently in axon diameters.

It is known from a series of recent studies in animals (for lit. see HEYMANS and NEIL, 1958 pp 72—76) that the baroreceptor activity is not only increased by distension of the carotid sinus, as effected by *e. g.* a blood pressure rise, but is also profoundly affected by changes in the intramural tension of the carotid sinus wall. Thus local application of drugs that increase vascular smooth muscle tone induces a considerable baroreceptor activity. It has even been suggested that there might be two types of baroreceptors, one of them connected to thin afferent fibres and functionally 'in series' with the smooth muscle cells, the other 'in parallel' with the muscle elements and in connection with thicker afferent fibres (LANDGREN 1952). It has also been reported that stimulation of the sympathetic branches to the carotid bifurcation can induce

an activation of the baroreceptors (PALME 1943, KEZDI 1954). Other investigators (FLOYD and NEIL 1952) could, however, only exceptionally find any evidence of such a mechanism. The functional organization of the baroreceptors is therefore still under debate with regard to their exact relationship to the muscle elements in the sinus wall. In six experiments in man the cranial sympathetic trunk, which should contain any existing constrictor fibres to the carotid sinus region, has been repeatedly stimulated at maximal rates and strength. In no case, however, did any reflex bradycardia or blood pressure fall occur which is in agreement with the largely negative results of FLOYD and NEIL (1952) in animal experiments. It is then more surprising that local application of noradrenaline, even in strong concentrations or given as an intramural infiltration, did not induce any significant reflex cardiovascular response in the two cases where it was tried. It can, however, not be excluded that the drug did not reach such parts of the sinus wall, where receptors influenced by increased smooth muscle tone are situated, and further experiments of this type are therefore needed.

Summary.

1. Direct stimulations of the carotid sinus nerve have been performed in man during operations of tumours in the neck. Definite reflex responses were obtained already at low stimulation rates, with maximal effects around 40—60 impulses per sec.

2. A reflex bradycardia and a blood pressure fall with a decreased pulse amplitude was obtained. Concomitantly there was a marked fall in resistance to blood flow within the forearm vascular bed.

3. The fall in pulse pressure is in all probability due to a decreased stroke volume, secondary to a reduced venous return when the capacity vessels are widened, and to an elimination of the positive inotropic effect on the heart, as a consequence of the generalized reflex inhibition of sympathetic tone.

4. Stimulation of the cranial sympathetic trunk gave no evidence of any activation of the carotid baroreceptors, secondary to increased intramural tension, as has been reported in some animal species.

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The Stimulation Threshold of Different Sympathetic Fibre Groups as Correlated to Their Functional Differentiation.

By

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It is well known that a distinct correlation exists between axon diameter and stimulation threshold on the one hand, and the functional differentiation of the fibres on the other, within the somatic nervous system. Thus motor fibres and fibres mediating impulses from muscle spindles have a bigger axon diameter, and hence lower stimulation threshold, than *e. g.* fibres mediating pain or the temperature senses. In a mixed somatic nerve it is therefore often possible to activate the different nerve fibre types more or less selectively by using suitable stimulation strengths.

MALTESOS and SCHNEIDER (1938 a, b, c) studied the activation of the autonomic fibres in sympathetic trunks by stimulations with alternating current, and observed that, with regard to their thresholds, the fibres showed a tendency to form relatively distinct groups. In all probability these threshold differences reflect differences in fibre diameter. The question then arises whether such differences in diameter of autonomic nerve fibres are correlated in a regular way to the functional differentiation of the neuroeffectors, as is generally the case within the somatic nervous system.

Methods.

The experiments were carried out on cats, anaesthetized with chloralose-urethane, 50 mg + 25 mg per kg of bodyweight. The following neuro-effector groups were studied: The nictitating membrane and the pupil with their nerve supply; the sympathetic vasoconstrictor control of the cutaneous arterio-venous anastomoses in the pads; the ordinary cutaneous vascular bed; the vessels in the skeletal muscles and the tongue; and lastly the sympathetic vasodilator fibres to the vessels of the skeletal muscles.

In the first series of experiments the characteristics of the sympathetic fibres to the vessels of the paw, including the specialized arterio-venous anastomoses within the pads, and the vasoconstrictor fibres to the skeletal muscles of the hind limb, were studied with special reference to their threshold to electric stimulation. The cats were eviscerated, leaving the liver, the stomach, the spleen and the kidneys intact. The right suprarenal gland was extirpated and the left one denervated in order to avoid any interfering release of blood-borne catechol amines. The two abdominal sympathetic trunks were then very carefully prepared free to allow electrical stimulations with a bipolar electrode at the level of the fourth lumbar vertebra, where all preganglionic fibres to the hind limbs have arrived from the spinal medulla, but where practically no sympathetic connections to lower parts of the limbs have as yet left the abdominal sympathetic trunks. For this reason practically all the sympathetic fibres to the regions studied could be simultaneously stimulated at this level. Just before the stimulations were to be started the sympathetic trunks were centrally cut in order to eliminate the tonic activity, and also to avoid possible reflex interferences, caused by stimulations of afferent fibres within the trunks. Further, atropine, 0.2—0.3 mg/kg was given to block the interfering effect of the sympathetic dilator fibres.

The animals were heparinized and the blood pressure was recorded from one of the carotid arteries by means of a mercury manometer. For a recording of the blood flow from the pads and adjacent skin regions the big saphenous vein of the left hind limb was cannulated just distally to the ankle joint, while adjacent venous collaterals were ligated in order to direct all the paw blood flow through the cannulated vein. The cannula was connected to a closed drop recorder unit, operating an ordinate writer, and the blood returned to the animal by way of a cannula in a branch of the left femoral vein. In the right hind limb the deep main branch of the femoral vein, which drains the muscles of the calf, was cannulated just above the knee joint, while a tight ligature just proximally to the ankle joint eliminated the circulation through the paw of this limb. In this way an almost pure muscle blood flow could be directed through another closed drop recorder unit, after which it was returned to the animal in a similar way as in the other limb. In some animals both the muscle and the skin blood flow were recorded from the same limb, and then only one of the abdominal sympathetic trunks was stimulated.

To allow stimulations of the two sympathetic trunks they were placed at exactly the same level on a bipolar electrode, after which they were insulated and protected by a paraffin oil bath, where the local temperature was kept around 37° C. Square-wave stimuli were delivered from a Grass stimulator, allowing variation of frequency, voltage and pulse-duration. In all experiments a series of stimulations was first performed in which the voltage and the frequency were kept constant with wide variations of the pulse-duration. Then followed another series where a constant pulse duration and frequency was maintained while the voltage was varied. These stimulation series were usually repeated several times in order to check the accuracy of the results. The frequency was, in most experiments, kept at 4 to 6 impulses per second to allow distinct and regular vascular effects to appear once all the fibres were excited. Lastly, a frequency-response correlation curve for the neuro-effectors under study was calculated from a series of recordings obtained by stimulation of the trunks with gradual increases of frequency from 1 impulse per second up to 20 impulses per second, while the voltage and pulse-duration were kept at supramaximal levels. A similar general procedure for sympathetic stimulation was used throughout in all the different types of experiments. In some experiments excitation of the sympathetic nerve trunks was induced by a stimulator designed for administering stimuli of constant current. This did not affect the results obtained, indicating that accidental shifts in nerve-tissue resistance can hardly have been of any significant influence. In order to distinguish between the sympathetic effects on the ordinary cutaneous vessels in the paw, and on the arterio-venous anastomoses, which are practically all confined to the pads, the circulation in the pads was obstructed by forceps, and the vascular effects of stimulation were studied both during obstruction and under normal conditions.

When the characteristics of the constrictor and dilator fibres to the vessels of the skeletal muscles were compared, the muscle blood flow was recorded as previously described, and the corresponding sympathetic trunk was prepared free for stimulation. In order to allow a strictly regional blockade of the vasoconstrictor nerve-endings a fine polyethylene catheter was introduced into the corresponding femoral artery with its tip located distally to the inguinal ligament, so that injections of an adrenergic blocking agent should only reach this limb. In this way the drug could not in significant amounts reach the fibres at the site of stimulation or the synapses between the pre- and postganglionic fibres, in case this might affect *e. g.* the stimulation threshold of the fibres which is a priori unlikely. After that a series of vasoconstrictor responses to sympathetic stimulation had been recorded, dihydroergotamine was slowly injected through the thin polyethylene catheter into the hindlimb, until a satisfactory blockade of its constrictor fibres had been obtained. No signs of a generalized sympathicolytic effect could be observed under such circumstances. The stimulations were then repeated at increasing intensities and impulse-durations until clearcut vasodilator responses had been obtained. Then

the cholinergic vasodilator fibres were also blocked by intraarterial injection of atropine and the stimulations were again repeated.

In another group of experiments the characteristics of the sympathetic vasoconstrictor control of the tongue vessels were compared with the nervous control of the nictitating membrane and the pupil. The venous outflow from one of the lingual veins was measured as previously described and the blood pressure was recorded from one of the femoral arteries. The contractions of the nictitating membrane on the same side were recorded in the conventional way with an 'isotonic' frontal writing-lever, and the corresponding cervical sympathetic trunk was carefully prepared free for preganglionic stimulation. The effects on the pupil were controlled only by inspection of the pupillary response.

Results.

Fig. 1 shows an experiment in which the cutaneous blood flow of the paw and the muscle blood flow in the calf were recorded while stimulations of the abdominal sympathetic trunks were administered. The figure shows first the responses obtained at a constant frequency and pulse-duration when the voltage was increased (A and B). Even at the voltage used in A the paw vessels react with a constriction that seems to be maximal for the frequency used, *i. e.* practically all the fibres to these vessels are excited (compare A and B). With regard to the vessels of the muscle, on the other hand, practically no effect is obtained at the voltage used in A, while the constrictor effect is marked in B. Therefore, in contrast to the case with the paw vessels, the stimulation strength used in A seems to excite only a minor fraction of the constrictor fibres to the muscles. It is possible, however, that this difference in reaction may merely be a consequence of the distribution of the different fibre-groups within the sympathetic trunk. If, for instance, the fibres to the muscle vessels were more centrally placed, the current at lower voltage would pass mainly through the fibres to the skin as they then would lie closer to the electrodes. Under such circumstances the fibres to the muscle vessels would be reached by the current flow first at a higher voltage. To exclude this possibility, the voltage was kept unchanged while the pulse duration was varied (see C, D, Fig. 1). As the same difference between the cutaneous and muscular vasoconstrictor responses reappeared, this evidently must be due to real differences in fibre threshold, and not caused by the spatial arrangement of the fibres. To exclude the remote possibility that the fairly similar cutaneous

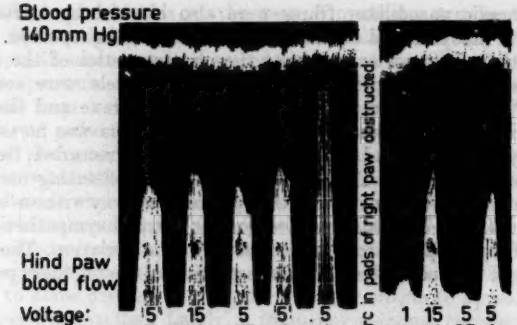

Fig. 1. Cat 3.8 kg. Chloralose-urethane. Records of blood pressure, blood flow in hind paw and calf muscles with effects of vasoconstrictor fibre stimulations at different pulse durations and voltage.

vasoconstrictor responses in A, B, C and D are due to the fact that these vessels reach their maximal constrictor capacity when only a fraction of their fibres are activated, the frequency was increased in E. A considerably stronger cutaneous vasoconstrictor response was then elicited. The pads were then clamped off, and the blood flow from the paw was now predominantly derived from other parts of the skin, where there are few, if any, arteriovenous anastomoses. This exclusion of the pad circulation naturally reduced the blood flow from the paw (to about 20 per cent; the ordinate-writer now being geared to a lower speed). Stimulations with similar characteristics to those in A, B, C and D were repeated (F, G, H, I, in Fig. 1), and it is obvious from the figure that the vasoconstrictor effects were now closely similar as to magnitude of response in relation to stimulation strength in both the skin and the muscles. Obviously the vasoconstrictor fibres to the muscles and the ordinary cutaneous vessels are closely related with regard to their stimulation threshold.

Lastly a few control experiments were performed. It might have been possible that the differences in threshold observed between *e. g.* the fibres to the arterio-venous anastomoses of the pads and the muscle vessels were a consequence of different locations of their synaptic relays within the sympathetic ganglionic trunk. If, for instance, the synapses of the fibres running to the arterio-venous anastomoses take place in more caudally located sympathetic ganglions — a priori unlikely due to the arrangement of the segmental innervation — the electrode might have throughout been in contact with their preganglionic nervous link, but with the postganglionic fibres to the muscle vessels. It is known that the preganglionic fibres have a lower threshold and a larger diameter than the postganglionic ones. To exclude this remote possibility as an explanation of the differences observed in threshold, stimulations were administered before and after a ganglionic blocking agent had been given intravenously. This drug almost completely abolished the constrictor response of both the pad vessels and the muscle vessels, which indicates that the electrode was only in contact with the preganglionic fibres of the two vascular regions.

Fig. 2 illustrates the differences in stimulation threshold of the sympathetic constrictor and dilator fibres to the muscle vessels. Firstly the sympathetic trunk was stimulated at a constant rate but with increasing pulse-duration and at a later stage also with increasing voltage until the maximally obtainable vasoconstrictor response for the frequency used was obtained. Then dihydroergotamine was given intraarterially to the limb to block selectively the constrictor nerve endings, and the sympathetic stimulation was repeated with gradually increasing strength and duration. A small constrictor response still appeared at the voltage and pulse duration range that had induced the maximally obtainable response before dihydroergotamine was given (compare C—D and E in Fig. 2). This was generally the case, as it is almost impossible to block the constrictor fibres completely with reasonable drug amounts. Even at this strength, however, which to judge from the first part of Fig. 2 is well above the threshold value for a considerable fraction of the vasoconstrictor fibres, no evidence of vasodilator fibre excitation appears. It was necessary further to increase the stimulation strength and pulse duration drastically (F in Fig. 2) to make a definite vasodilator response appear. In G, Fig. 2, the sympathetic trunk was stimulated con-

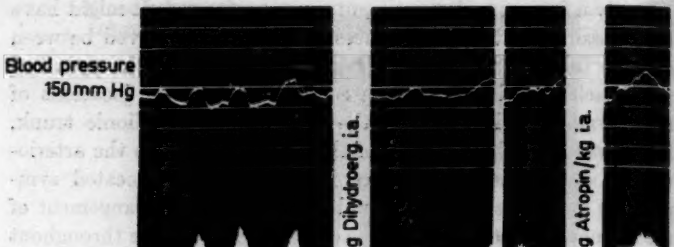

Fig. 2. Cat 3 kg. Chloralose-urethane. Records of blood pressure and blood flow in calf muscles. Effects of sympathetic stimulation at different pulse durations and voltage before and after a pharmacologic block of the vasoconstrictor fibres and the cholinergic vasodilator fibres.

tinuously at a given frequency, while the voltage and the pulse duration were gradually raised. It can then be seen that first a weak vasoconstrictor response appears as an effect of the constrictor fibres being incompletely blocked, but suddenly, when the voltage is further increased, the vasoconstrictor effect is replaced by a clearcut vasodilator response, obviously due to the fact that now for the first time the threshold of the cholinergic dilator fibres is reached. The fact that the dilator response is again eliminated by 0.2 mg atropine given intraarterially to the limb, and is then replaced by a vasoconstriction supports the view that it was caused by the cholinergic vasodilator fibres (H in Fig. 2). In some of these experiments where the blood flow from the muscles of the other limb was also recorded and the corresponding sympathetic trunk stimulated, it was possible to check that the threshold of the constrictor fibres of this limb was not changed by the regional injection of the blocking agents.

In a third group of experiments, illustrated in Fig. 3, the stimulation thresholds of the fibres to the nictitating membrane, and the vasoconstrictor fibres to the tongue vessels, were compared. Here also the voltage and the frequency were first kept constant while

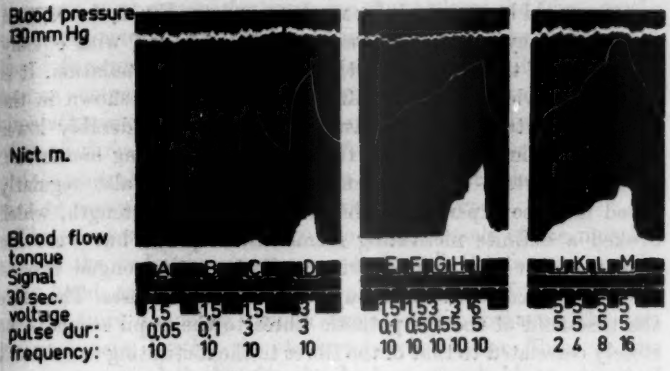

Fig. 3. Cat 3.5 kg. Chloralose-urethane. Records of blood pressure, nictitating membrane contractions and blood flow in the tongue with effects of sympathetic stimulations at different pulse durations and voltage.

the pulse-duration was gradually increased. It is obvious from the figure (A—C) that the fibres to the nictitating membrane have on average a considerably lower stimulation threshold than those to the vessels of the tongue, as the pulse-duration necessary to excite the fibres to the nictitating membrane must be considerably increased before any vasoconstrictor response appears in the tongue. Only when the voltage also was increased did the vasoconstriction in the tongue become pronounced (D in Fig. 3). In E-I, Fig. 3, the pulse-duration and voltage were gradually increased during a continuous stimulation at a constant frequency. At a point where, as yet, no response of the tongue vessels is seen (G), the contraction of the nictitating membrane is not far from its maximum. There remained the possibility, however, that the difference between the tongue and the nictitating membrane might be explained as the result of a considerably steeper 'dose-response' curve for the nictitating membrane, so that it responded with a proportionally much larger contraction than the tongue vessels to small concentrations of the mediator substance. To exclude this alternative explanation of the findings shown in the first two parts of Fig. 3, the sympathetic trunk was stimulated at a strength and for a duration that caused all fibres to be excited. The frequency was then gradually increased from 2 impulses per second up to 16 impulses per second, so that the relationship between the rate of stimulation and smooth muscle response for the two neuro-effector

systems could be obtained. It may be seen from Fig. 3, J—M, that their frequency-response curves are closely similar, with equally steep rises of the response at the lower rates of stimulation. It is therefore obvious that the differences in response shown in the first two parts of Fig. 3 must be due to a considerably lower stimulation threshold for the fibres to the nictitating membrane, though a certain overlap seems to exist. It was also regularly noted in these experiments that the stimulation strength, which evoked a definite nictitating membrane response but were too weak to elicit any vasoconstrictor effect on the tongue vessels, always induced pronounced pupillary dilator responses. Therefore the threshold of the sympathetic fibres to the pupil seems to be closely correlated to that of the fibres to the nictitating membrane.

Discussion.

In the present experiments the preganglionic sympathetic fibres to a number of functionally different neuro-effector groups have been given carefully graded stimulations. Interest has been concentrated on the fibres to the nictitating membrane and the pupil, the constrictor fibres to the arterio-venous anastomoses of the foot pads, the ordinary skin vessels, the muscle and the tongue vessel, and, lastly, also on the sympathetic dilator fibres to the muscle vessels. By means of various experimental modifications, previously described in detail, evidence has been presented to indicate that relatively marked differences in the threshold to electric stimulation exist for the preganglionic sympathetic fibres to the various regions. Such differences in threshold are, by earlier neurophysiological studies, known to be correlated to corresponding differences in fibre diameter. These differences in threshold, which thus reflect differences in fibre diameter, show a distinct and regular correlation to differences in the functional differentiation of the sympathetic neuro-effector groups studied. The fibres to the arterio-venous anastomoses of the pads, to the nictitating membrane, and to the pupil thus seem to have on average bigger fibre diameters than is the case for the other groups of sympathetic neuro-effectors studied. The characteristics of the three autonomic neuro-effectors mentioned are that they are especially strongly dominated by their central nervous control and that they consequently show very little myogenic automaticity (see FOLKOW 1955). Further,

there are good reasons for assuming that at least the fibres to the cutaneous arterio-venous anastomoses, specialized to subserve the heat-loss centre in warm-blooded animals, appear late in evolution as compared with, *e. g.*, the constrictor fibres to the systemic circulation in general. In some respects the functional characteristics of these three autonomic neuro-effectors, with their almost completely centralized control, are more related to the skeletal muscles than to many of the visceral smooth muscle types.

The vasoconstrictor fibres to the blood vessels of the muscles, and to the ordinary cutaneous vessels and the tongue, on the other hand, seem to form another group with a definitely smaller average fibre diameter; though some overlap between the two groups exists. Lastly, the cholinergic vasodilator fibres to the muscle vessels show by far the highest threshold to electrical stimulation, and therefore seem to form a third sympathetic fibre group with still smaller average axon diameters.

These results thus indicate that there exists a definite correlation between stimulation threshold and axon diameter on the one hand and the functional organization on the other also within the autonomic nervous system, as is known to be the case within the somatic nervous system. It seems, however, as if the different sympathetic neuro-effectors show a considerable overlap with regard to their stimulation thresholds, and probably differences in fibre diameters are by far not so marked as is the case within the somatic nervous system, where, *e. g.*, the diameters of fibres mediating deep sensitivity are far larger than those of the pain fibres.

Summary.

1. The differences in stimulation threshold of the preganglionic fibres to a number of functionally-differentiated sympathetic neuro-effectors have been studied.

2. The results indicate that the fibres to the cutaneous arterio-venous anastomoses, to the nictitating membrane and the pupil have as an average the lowest threshold, and hence probably the largest fibre diameters. Another group is formed by the constrictor fibres to, *e. g.*, the ordinary skin vessels, the muscle and the tongue vessels, while the sympathetic dilator fibres to the muscle vessels have by far the highest threshold, and therefore probably the smallest diameter.

This work has been carried out with the aid of the Swedish Medical Research Council.

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Inhibitory Action of Allicin on Degranulation of Mast Cells Produced by Compound 48/80, Histamine Liberator from Ascaris, Lecithinase A, and Antigen.

By

BERTIL HÖGBERG and BÖRJE UVNÄS.

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HÖGBERG and UVNÄS (1957) recently presented experimental evidence that 48/80 degranulates mast cells by activating an enzyme mechanism. A lytic enzyme was thought to be attached to the mast cell membrane. The enzyme, it was suggested, carried essential amino groups since the degranulation of the mast cells was blocked by acetic anhydride and this inhibition was not reversed under conditions which hydrolyzed phenyl acetate and thiol acetate. This conclusion was supported by the observed inactivation of the enzyme by formaldehyde and phenylisocyanat and several other reagents under conditions where these reagents are fairly specific for protein amino groups.

Many enzymes are known to depend on SH groups for their activity. In this study evidence is presented which indicates that the enzyme mechanism activated by 48/80 also is dependent on SH groups, as is the degranulation of mast cells produced by lecithinase A, by a histamine liberator extracted from *Ascaris lumbricoides* (eelworm of swine), and by antigen (horse serum) in sensitized rats.

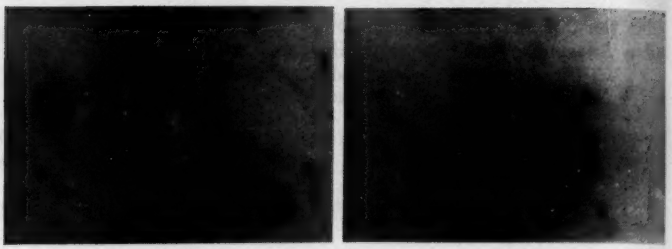

Fig. 1. Normal (left) and sensitized (right) rat mast cells incubated with horse serum 1 : 1,000.

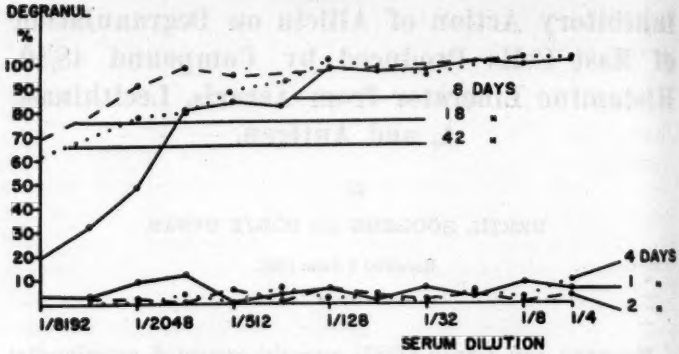

Fig. 2. Development of sensitization of rat mast cells to horse serum.

Methods.

Technique for Observations on Mast Cells. A modification of the technique described by HÖGBERG and UVNÄS (1957) was used. The cells were incubated in a solution containing NaCl 0.9 per cent, KCl 0.02 per cent, $CaCl_2$ 0.01 per cent and 10 per cent Sörensen phosphate buffer at pH 7.4.

Sensitization of Rats. The rats were sensitized to horse serum by a subcutaneous injection of 0.5 ml horse serum mixed with 1.0 ml ($2 \cdot 10^{10}$ bacilli) pertussis vaccine. In the presence of this vaccine a marked sensitivity of the mast cells to horse serum developed within 10 days (Fig. 1 and 2) and persisted for at least six weeks.

Histamine Liberator from Ascaris Lumbricoides. A highly active liberator was obtained from alcoholic extracts of the worms by ion exchange chromatography on Amberlite IRC-50 (XE-64). The purification procedure and the characteristics of the liberator will shortly be reported in this journal.

Compound 48/80 was prepared according to the method described by BALTZLY, BUCK DE BEER and WEBB (1949).[1]

Allicin (Fig. 3) was prepared from garlic according to the technique described by CAVALLITO and BAILEY (1944).

Allicin

$$CH_2 = CH - CH_2 - S - S - CH_2 - CH = CH_2$$
$$\|$$
$$O$$

Fig. 3. Chemical structure of allicin.

Lecithinase A was prepared from bee venom by ion exchange chromatography on Amberlite IRC-50 (XE-64) by a method of HÖGBERG (unpublished).

Results. Degranulation of mast cells, produced in four different ways, was in each instance blocked by allicin in very low concentrations (Fig. 4).

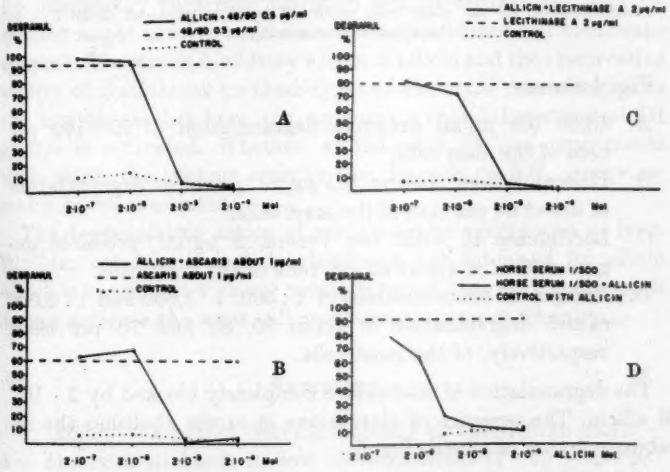

Fig. 4. Inhibitory action of allicin on degranulation of mast cells produced by 48/80; histamine liberator from Ascaris, Lecithinase A and antigen (horse serum). For explanation see text.

[1] We are indebted to Dr. H. FEX, of the Leo Research Laboratories, for preparing compound 48/80.

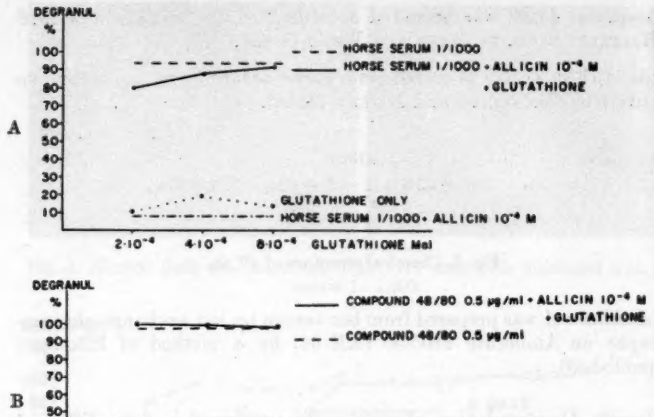

Fig. 5. Reversal with glutathione of the inhibitory action of allicin on degranulation of rat mast cells produced by A) compound 48/80 B) antigen (horse serum).

Fig. 4 shows:

A. 48/80 0.5 µg/ml produces degranulation of 90—100 per cent of the mast cells.

B. Liberator from *Ascaris*, 0.5 µg/ml, produces degranulation of about 60 per cent of the mast cells.

C. Lecithinase A from bee venom, 2 µg/ml, produces degranulation of about 80 per cent of the mast cells.

D. Antigen in concentrations of 1 : 500, 1 : 1,000 and 1 : 2,000 causes degranulation of about 90, 85 and 70 per cent, respectively, of the mast cells.

The degranulation of mast cells is completely blocked by $2 \cdot 10^{-5}$ M allicin. The presence of glutathione in excess abolishes the inhibitory action of allicin (Fig. 5).

Discussion.

Extracts of *Allium sativum* contain a powerful bactericidal agent, allylthiosulfinic allyl ester (allicin). WILLS (1956) showed

that allicin in concentrations around 10^{-5}—10^{-4} M inhibits many sulfhydryl, but very few non-sulfhydryl, enzymes of twenty-eight tested. HÖGBERG and UVNÄS (1957) had earlier presented evidence that 48/80 causes degranulation of mast cells by activating a lytic enzyme attached to the mast cell membrane. The fact that specific acetylation and phosphorylation of amino groups blocked the degranulating action of 48/80 and other positively charged polymer liberators suggested that amino groups were essential for the enzyme activity. The powerful inhibitory action of allicin and the restoring ability of glutathione tends to show that the enzyme activity is dependent on SH groups too.

The susceptibility of lecithinase A to all the inhibitors that were found to block the action of 48/80 — including specific acetylation and phosphorylation — led us to surmise that the enzyme taking part in the degranulation of mast cells might be some kind of a lecithinase. The observation that lecithinase A was strongly inhibited by allicin (total inhibition by $2 \cdot 10^{-5}$ M allicin) strengthened this hypothesis.

We further suggested that the mast cell degranulation caused by biologically occurring liberators and by antigen-antibody reactions might involve activation of the postulated cell membrane enzyme. The strong inhibitory action of allicin and the reactivating ability of glutathione on these types of histamine release supports the hypothesis that here, too, an enzyme containing essential SH groups is activated. Whether, as indicated by our experiments with 48/80, the enzyme activity also depends on NH_2 groups remains to be elucidated.

The degranulating action of surface-active agents such as lysolecithin, octylamine and decylamine is not inhibited by allicin which is consistent with our previous report that such compounds do not activate the mast cell membrane enzyme mechanism.

Summary.

1. Allicin — a potent enzyme inhibitor prepared from garlic — was observed to block in low concentrations ($2 \cdot 10^{-4}$—$2 \cdot 10^{-4}$ M) the *in vitro* degranulation of rat mast cells produced by compound 48/80, by a histamine liberator prepared from *Ascaris lumbricoides*, by antigen-antibody reaction, and by lecithinase A. Glutathione in excess reversed the blocked degranulating processes.

2. The observations were considered to support our theory that the degranulation of mast cells produced by those agents is due to activation of a lytic enzyme attached to the mast cell membrane.

3. This enzyme is — in common with lecithinase A — dependent on sulfhydryl groups for its activity.

This investigation was supported by The Harald and Greta Jeansson Foundation and The Gustaf and Tyra Svensson Memorial Foundation.

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On the State of the Catechol Amines of the Adrenal Medullary Granules.

By

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Apart from the granule membrane, the four main constituents of the specific adrenal medullary granules are the catechol amines (chiefly adrenaline and noradrenaline), adenosine triphosphate (ATP), a protein, and water. The catechol amines and ATP are present in almost equivalent proportions (HILLARP 1958 a). At stimulation of the adrenal medulla *via* the splanchnic nerves, the catechol amines and ATP are released, whereas most of the protein seems to remain inside the essentially intact granule membrane (CARLSSON and HILLARP 1956, CARLSSON, HILLARP and HÖKFELT 1957).

In the present paper it is shown that the catechol amines are present in the granules in an osmotically inactive form. The granule membrane was found to be permeable to sodium, potassium, sucrose, and catechol amines.

Methods.

Isolation of the Medullary Granules. The catechol amine containing granules of the cow adrenal medulla were isolated in 0.3 M sucrose by means of differential centrifugation as described in a previous paper (HILLARP 1958 b). The procedure gives a fraction which is but slightly contaminated by mitochondria and microsomes.

Determination of Total Cationic Content. In order to denaturate the protein and prevent breakdown of ATP, the granules (containing about 120 μM catechol amines) were heated for 9 minutes in boiling water. A water extract of the granules was passed through a cation exchange column (Dowex 50, 150—300 mesh, hydrogen form, 40 × 8 mm). The column was washed with water. The combined effluents were titrated with 0.1 N NaOH to the same pH (about 6.6, determined by glass electrode) as had the original extract after dilution to the same degree as the combined effluents. The fact that an additional 5 per cent of the amount of NaOH used caused a rise in pH of 0.2 to 0.3 units, may serve to illustrate the accuracy of the determinations. Blank titrations were performed. The small values thus obtained were subtracted from the values obtained by titration of the effluents.

Permeability Studies. A. Sodium, Potassium and Sucrose. Medullary granules were suspended at 0° C in 0.15 M NaCl, 0.15 M KCl, or in sucrose solutions containing various concentrations of NaCl or KCl. After one hour the granules were spun down. Wet and dry weights (constant weight over P_2O_5 at room temperature) of the granule pellet were determined. The sodium, potassium, and sucrose contents of the pellet and the supernatant were determined.

B. Catechol Amines. Medullary granules were suspended at + 4° C in 0.2 M l-adrenaline or dl-noradrenaline (the pH of solutions of the hydrochlorides was adjusted to 6.5 with NaOH). Parallel controls in 0.3 M sucrose were run. After 18 hours the granules were spun down. The granular pellet was weighed. The adrenaline and noradrenaline contents of the pellet and the supernatant were determined.

Determination of Extragranular Water of the Granule Pellets. Medullary granules were suspended in 0.3 M sucrose. To 3 ml suspension 5 ml 0.2 M KCl, containing 5 per cent dextrane (molecular weight 197,000), was added. After mixing, the granules were spun down as above (38,000 × *g* for 60 min at 0° C). The supernatant was removed as completely as possible. The dextrane contents of the pellet and the supernatant were determined as described below.

Radioiodinated serum albumin could not be used for this purpose, since the granules were found to take up considerable amounts of radioactive material.

Analytical Methods. Adrenaline and noradrenaline were determined according to the colorimetric method of EULER and HAMBERG (1949). Sodium and potassium were determined in trichloracetic acid extracts of the samples, using a Beckman spectrophotometer Model DU with a flame assembly. Sucrose was separated from catechol amines by cation exchange and then quantitated by means of the anthrone reagent according to FALES (1951). The same method was used for determining dextrane, according to the following procedure. The weighed pellets were extracted four times with 80 per cent methanol, which removed sucrose and other interfering material. Dextrane is insoluble in 80 per cent methanol. The residues were extracted with 5 per cent trichloracetic acid and made up to 10 ml. The extracts were assayed by means of the

Table I.

Catechol Amine and Total Cationic Content of Water Extract of Medullary Granules Isolated From 0.3 M Sucrose.

The data are given in microequivalents.

Experiment No.	Catechol Amines	Total Cations	Catechol Amines / Total Cations
1	78	79	0.99
2	73	76	0.96
3	82	87	0.94
4	89	92	0.97
5	82	85	0.97
6	82	83	0.98
		Average	0.97

anthrone reagent, using dextrane as standard. Sensitivity: 5 μg. Recoveries of dextrane added to granules: 102 and 104 per cent. The dextrane of the supernatants was precipitated by addition of methanol to yield a final concentration of 80 per cent. The precipitates were washed with 80 per cent methanol, dissolved in 5 per cent trichloracetic acid, and assayed by means of the anthrone reagent.

Results.

Ionic Composition of Water Extracts of Medullary Granules Isolated from 0.3 M Sucrose. The catechol amines were found to constitute about 97 per cent of the total cations of the extracts (Table I). As ATP and the catechol amines are present in the granules in about equivalent proportions, it follows that ATP, provided its breakdown is prevented, would be the main anionic constituent of water extracts of granules isolated from sucrose.

Permeability of Granules to Sodium, Potassium, and Sucrose. The results of experiments in which granules were suspended in solutions of varying ionic composition, are summarized in Figure 1. It is evident that the composition of the granule fluid reflects that of the suspension medium. It appears that the granule membrane is permeable to sodium, potassium, and sucrose.

The data of Figure 1 were calculated on the assumption that all the water of the granule pellets was intragranular. Obviously

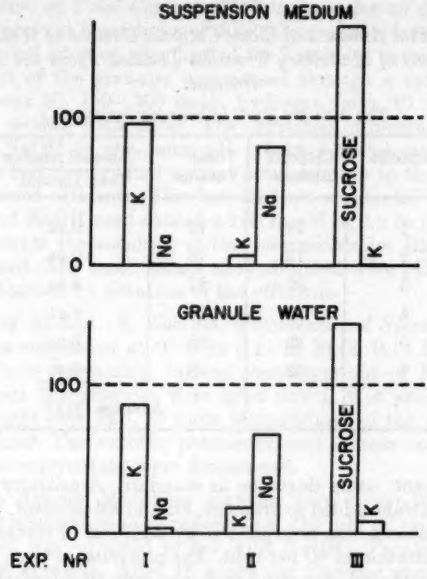

Fig. 1. Sodium, Potassium, and Sucrose Concentrations in Granule Water after Suspension of the Granules in Solution of Varying Ionic Composition.
The level at 100 indicates a concentration of 0.15 N (sodium, potassium) or 0.3 M (sucrose).

this assumption is not quite correct, owing to the presence of some suspension fluid between the granules. An estimate of the amount of extragranular fluid of the pellets was obtained by isolating granules from suspension fluids containing dextrane and determining the dextrane content of the pellets. In this way the extragranular water of the pellets was estimated to constitute about 15 per cent of the total pellet water (Table II). This fairly small amount does not invalidate the conclusion that the composition of the intragranular fluid reflects that of the suspension medium.

Permeability of Granules to Catechol Amines. Granules isolated from suspension media containing adrenaline or noradrenaline contained more catechol amines than granules isolated from sucrose solutions (Table III). The concentration of this additional adrenaline and noradrenaline in the granule water was calculated to be

Table II.

Amount of Extragranular Water in Granule Pellets.

Experiment no.	Total water in pellet ml	Dextrane in pellet ml	Dextrane in susp. medium mg/ml	Extragranular water	
				ml	per cent total
1	0.448	2.33	35.2	0.066	14.7
2	0.400	2.06	34.4	0.060	15.0
3	0.600	3.14	33.5	0.094	15.7

Table III.

Adrenaline (A) and Noradrenaline (NA) in Granules Isolated From Solutions of A and NA.

		Granules isolated from a solution of		
		A	NA	Sucrose
A in granules, mg	Total	8.0	4.8	5.0
	Increase	3.0	—0.2	—
NA in granules, mg	Total	2.3	4.9	2.2
	Increase	0.1	2.7	—
Increase in A in granules, moles per litre granule water		0.15	—	—
Increase in NA in granules, moles per litre granule water		—	0.17	—
A in supernatant, moles per litre		0.17	—	—
NA in supernatant, moles per litre		—	0.17	—

about the same as the concentration of the amines in the suspension medium. When granules isolated from suspension media containing adrenaline and noradrenaline were resuspended in sucrose solutions, it appeared that the additional catechol amines were again lost to the suspension medium (Table IV). Thus granules which had been isolated from adrenaline solutions, lost adrenaline but little noradrenaline on resuspension in sucrose, whereas granules isolated from noradrenaline solutions gave off noradrenaline but little adrenaline. This suggests that catechol amines added to the suspension medium can move between this medium and the granule water without mixing appreciably with preexisting intragranular catechol amines.

Table IV.

Reversal of Adrenaline (A) and Noradrenaline (NA) Uptake In Vitro by Granules on Resuspension in 0.4 M Sucrose.

Granules which had been suspended for 18 hours in 0.2 M adrenaline, 0.2 M noradrenaline, or 0.3 M sucrose, were isolated and resuspended in 0.4 M sucrose for 3 hours at + 4° C. They were then isolated, and granules and supernatants were analysed for A and NA.

	Granules previously suspended in a solution of		
	A	NA	Sucrose
Adrenaline, mg			
Granules	9.7	7.7	9.0
Supernatant	4.7	0.5	0.35
Total	14.4	8.2	9.4
Noradrenaline, mg			
Granules	2.4	3.9	3.3
Supernatant	0.5	4.3	0.15
Total	2.9	8.2	3.5

Discussion.

The data presented above indicate that the granule membrane is permeable to sodium, potassium, sucrose, and catechol amines. It might be argued that since the medullary granules readily undergo lysis in distilled water, the granule membrane cannot be freely permeable to ions. However, the kinetic aspect of the lysis phenomenon has to be considered. Lysis may be due to the fact that water passes membranes more rapidly than do most other ions and molecules.

The osmotic pressure of the intragranular fluid appeared to be maintained by the same constituents as in the suspension media. If the osmotic activity of the preexisting intragranular catechol amines and ATP were at the theoretical maximum level, these constituents alone would maintain an osmotic pressure at least twice that of an isotonic saline solution (Hillarp 1958 a). The conclusion seems inescapable that the catechol amines occur in the granules in an osmotically inactive form, probably in combination with an equivalent amount of ATP.

If medullary granules undergo lysis in distilled water, the catechol amines and ATP are promptly released and then seem to

occur as ions in true solution. For example, they are dialysable and are easily taken up by cation and anion exchangers, respectively. It does not seem very likely that they occur in the granules in a crystalline form. We have evaporated the diluted intragranular fluid obtained by lysis in distilled water and subsequent removal of the granule residues by centrifugation. The original water content of the intragranular fluid was thus restored. This procedure yielded an extremely viscous, optically homogenous fluid, readily miscible with water. No precipitation of catechol amines was detectable. It does not seem unlikely that in this way the physiologic state of the intragranular fluid was restored.

Summary.

Catechol amine containing granules of the cow adrenal medulla were isolated and resuspended in solutions of varying ionic composition. Analyses of the intragranular fluid indicated that the granule membrane is permeable to sodium, potassium, sucrose, and catechol amines. It was concluded that the catechol amines occur in the granules in an osmotically inactive form, probably in combination with an equivalent amount of adenosine triphosphate.

This investigation was aided by grants from the Swedish State Medical Research Council. The authors are indebted to Mr. T. Magnusson for carrying out the sodium and potassium analyses.

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Experimental Demonstration of the Euclidean-Pythagorean Structure and the Quadratic Metrics in the Perceptual Manifold of the Cutaneous Tactile Sense.

By

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Introduction. In his immediate observation of phenomena in the external world, man is able to define quantities of the observed object by means of assessing "subjective" perceptional equivalencies and differences in the spatial, temporal, intensity and qualitative relations of this object. Since on the other hand the quantitative relations of the object may also be defined by means of measurement employing conventional physical expedients, we have thus an opportunity to compare mutually the results of immediate observation, *i. e.* of "subjective" measuring, and of physical measurement. This implies that we have a means of obtaining knowledge of the metrics prevailing in the particular "perceptual manifold" (REENPÄÄ 1953, 1958) to the phenomena of which the immediate, measuring, observation has to be made. According to REENPÄÄ's theoretical analysis (1952, 1953), the perceptual manifold can be represented in a uni-original coordinate system, its dimensions (the above-mentioned: time, place, intensity and quality) being mutually independent and therefore constituting an orthogonal system, which implies that the pro-

jection of each dimension upon any other dimension is equal to zero. By pure theoretical reasoning, REENPÄÄ has assumed that the perceptual manifold possesses a Euclidean-Pythagorean, linear structure where quadratic metrics apply; this has found experimental support in the optic sensory domain (BERGSTRÖM and REENPÄÄ 1957).

Problem and associated theory. Since, originally, the Euclidean-Pythagorean structure of the percept presumed in general and experimentally proved in the optical sensory domain (optical modal sphere), and the quadratic metrics applying in it had been derived with consideration of the intermodal structure (REENPÄÄ 1952, 1953), it seems reasonable to surmise that it should be demonstrable also in other sensory domains than the optical. Since, however, representation of the perceptual manifolds in accordance with the said structure is equivalent to a certain kind of geometrisation of the perceptual sphere of phenomena and this "geometrical character" is typical particularly of the optical modal sphere, it is to be expected that experimental demonstration of this structure and of the metrics applying in it may also succeed in the modal sphere of skin sensation (the haptic modal sphere), which resembles the optical sensory domain in many respects.

In an attempt to demonstrate experimentally the Euclidean character of some two-dimensional (x, y) component structure of the percept concerned in the act of observing and the quadratic metrics applying in it, the metric expression to be proved will be the following equation composed of vector quantities:

$$|\vec{s}| = \sqrt{|\vec{x}|^2 + |\vec{y}|^2} \tag{1}$$

where $\vec{s}$ can be interpreted as a percept vector in a coordinate system, with the components $\vec{x}$ and $\vec{y}$ parallel to the coordinate axes. Without entering into the theoretical basis of this equation (reference should be made here to the investigations cited above), we shall study in the following the implications arising from it with regard to the experimental arrangement of the present investigation.

For the present study, the component space (possessing surface area and intensity dimensions), of this sensory sphere was chosen in analogy with the above-mentioned investigation performed in the optical sphere. Denoting the dimension of surface area with

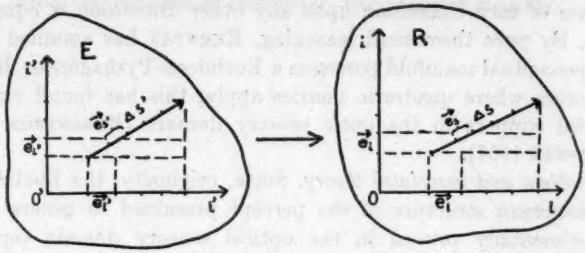

Fig. 1. The haptic component structure with intensity (i) and surface area (l) dimensions, shown as a relation of mapping ($\rightarrow$) between the perceptual manifold (E) and the stimulus manifold (R).

l and that of intensity with i, the equation to be empirically proven will be

$$|\vec{\Delta s}| = \sqrt{|\vec{\Delta i}|^2 + |\vec{\Delta l}|^2} \tag{2}$$

In this equation $|\vec{\Delta s}|$ stands for the amount of such a change of a percept vector which is concerned when a change occurs in the sensation simultaneously with regard to touch intensity (i) and to touch area (l).

In Fig. 1, the component structure E with intensity and surface area dimensions of the perceptual manifold under investigation has been represented by a coordinate system (i', l'), in which the change observed by the subject is shown as a vector $\vec{\Delta s}'$. The change has been brought about by means of a change of stimulus in the stimulus sphere R, this latter being represented by a vector $\vec{\Delta s}$ in the coordinate system with intensity and surface area dimensions (i, l) of the stimulus manifold. The length of the vector $\vec{\Delta s}$ in the stimulus sphere can be determined from the conditions of stimulus in the desired, arbitrary units. Thus, one may say that to the vector $\vec{\Delta s}'$ in the E sphere corresponds the vector $\vec{\Delta s}$ in the R sphere, and this fact is expressed by the arrow ($\longrightarrow$), which we call mapping, which has been placed between the E and R spheres.

In order that the numerical values of the vector lengths involved in equation (2) might be defined in such a manner that

they will represent the metrics of the percept, it is necessary to base this definition on the perception itself. This is made possible by the fact that the perceptual manifold contains in itself, in the form of distinction thresholds, a natural system of measurement based on perception (REENPÄÄ 1952). *The magnitude of any change that has occurred in the percept is thus definable as a number n expressing the number of distinction thresholds contained in this change (and thus, according to its original definition, an integer number).*

Let the change of experience in the direction of the *l* coordinate be denoted by the vector $\vec{\Delta l}$ and a change in the direction of the *i* coordinate by the vector $\vec{\Delta i}$, and let $\vec{\Delta s}$ stand for a change taking place simultaneously in both dimensions being expressible as the sum of its components *l* and *i*. If the numbers n_l, n_i and n_s are employed to indicate the respective number of "unit vectors" $(\vec{e})$, representing the distinction thresholds, contained in these vectors, we may use the following expressions to represent the percept vectors:

$$\vec{\Delta l} = n_l \cdot \vec{e_l}, \tag{3}$$

$$\vec{\Delta i} = n_i \cdot \vec{e_i}, \tag{4}$$

$$\vec{\Delta s} = n_s \cdot \vec{e_s}. \tag{5}$$

If the vectors $\vec{e_l}$, $\vec{e_i}$, $\vec{e_s}$ representing the distinction thresholds are considered as unit vectors and their lengths are agreed to have the numerical value 1, then is

$$|\vec{e_l}| = |\vec{e_i}| = |\vec{e_s}| = 1. \tag{6}$$

Consequently, the lengths of the vectors represented by equations (3), (4) and (5) can be given as

$$|\vec{\Delta l}| = |n_l|,$$
$$|\vec{\Delta i}| = |n_i|,$$
$$|\vec{\Delta s}| = |n_s|,$$

and the expression to be demonstrated experimentally is, from equation (2),

$$n_s = \sqrt{n_i^2 + n_l^2} \tag{7}$$

According to the foregoing, it is possible in the investigation to be described below to define the numerical values of the quantities occurring in equation (2) in the perceptual manifold itself. On the other hand that which is defined on the basis of the percept in the perceptual manifold can be mapped in the sphere of the stimulus in arbitrary, physical systems of measuring units. It is then possible, by observing the number of distinction thresholds contained in the percept vectors in the perceptual manifold *E* (Fig. 1) and by mapping the vector transpositions that have occurred in the *E* sphere by means of the numerical values observed in the *R* sphere, to study the validity of quadratic metrics in the sphere of percept.

Methods. The subject was placed in an easy and comfortable sitting position at a desk. His right arm rested on a soft base, with his hand supported in a plaster mould with slightly clenched fist and the thumb resting securely in a horizontal depression, its tip protruding freely from the mould. In the tests, impacts were directed upon the sensitive volar surface of the distal phalanx of the thumb, by means of a pendulum of accurately known physical dimensions. To the mass of the pendulum, capstans of equal weight could be attached, so that the surface of contact with the thumb at the moment of impact could be varied between 1 and 100 mm². Contact occurred simultaneously all over the surface of the capstan and at the moment when the pendulum passed through its rest position. The force of impact was regulated by means of the initial deflection given to the pendulum before it was released. This deflection, in degrees, could be read from a gratuated plate attached to the pendulum support. The physical characteristics of the pendulum being known, the impulse delivered by it to the skin of the thumb could be computed; the calculations were also checked empirically by determining the velocity of passage through the rest position of the pendulum for various initial deflections. In the tests, impulses varying in magnitude between 600 and 1,300 c. g. s. units were employed.

The subject was asked in these tests to determine just barely perceptible differences, *i. e.* distinction thresholds, under the following sets of conditions: 1) with relation to impact force ($\vec{e_i}$) at constant contact surface area, 2) with relation to contact surface area ($\vec{e_l}$) at constant impact force, and 3) with relation to both impact force and contact area, *i. e.* with relation to their simultaneous perception ($\vec{e_s}$).

The procedure was firstly to deliver a reference impact with impact force and contact area of desired magnitude (in the present tests 656.8 c. g. s. units and 1 mm², respectively), the values considered optimal from the viewpoint of the perception. After this, another impact was delivered as a comparison, its force or contact area or both simultaneously being such as to make the impact exceed that of the reference

value in magnitude. The subject was required to state whether or not he could observe any difference between the impacts. The magnitude of successive impacts was increased in sufficiently small, sub-threshold steps so that the distinction threshold could be determined with an adequate accuracy. As a rule, a barely perceptile difference was achieved after 3 to 4 alterations. Another determination of distinction threshold was then instituted, choosing as reference point the impact value from the preceding determination which was just sufficient to produce a perceptible difference in percept. In this manner it was possible to proceed, from a chosen magnitude of impact force and contact area, by distinction threshold steps through any required number of steps either in the dimension of impact force or that of contact area, or in both dimensions simultaneously.

A number of test series of the following type were carried out according to this procedure, in which the dimension of the particular threshold under determination was varied, from one determination to the other, in the following manner:

$$
\begin{array}{ll}
A & \vec{e}_{i1}, \vec{e}_{l1}, \vec{e}_{i2}, \vec{e}_{l2}, \ldots \vec{e}_{in}, \vec{e}_{ln}; \\
B & \vec{e}_{l1}, \vec{e}_{i1}, \vec{e}_{l2}, \vec{e}_{i2}, \ldots \vec{e}_{ln}, \vec{e}_{in}; \\
C & \vec{e}_{l1}, \vec{e}_{l2}, \ldots \vec{e}_{ln}, \vec{e}_{i1}, \vec{e}_{i2}, \ldots \vec{e}_{in}; \\
D & \vec{e}_{i1}, \vec{e}_{i2}, \ldots \vec{e}_{in}, \vec{e}_{l1}, \vec{e}_{l2}, \ldots \vec{e}_{ln}; \\
E & \vec{e}_{s1}, \vec{e}_{s2}, \ldots \vec{e}_{sn}.
\end{array}
$$

In this representation of the series, $\vec{e}$ stands for the percept unit vector determined by the distinction threshold, its first subscript (l, i, s) indicating the dimension in which vectorial transposition has taken place (l = contact area, i = impact force, s = both simultaneously), while the second subscript (1, 2, . . ., n) gives the consecutive number of the particular threshold value in the temporal sequence of the series.

In accordance with the problem in hand as expressed by equations (2) and (7), several series of type E were performed with each test subject in one session, and one of the series A, B, C or D. It was then possible to determine from the E series the numerical value of n_s in equation (7) and from the other series the n_i and n_l values occurring in the square root expression. Most tests were performed as a combination of the E and C series.

Tests were made with 12 subjects, altogether 20 C series and 35 E series being performed with them. Moreover, five subjects participated in tests with altogether five each of the A, B and D series. The subjects were mainly students of medicine (between 18 and 25 years of age).

Results. Fig. 2 shows a typical result from series of the types A, B, C and D performed with subject A. All series were performed in one session. The abscissa axis represents the dimension of the contact surface (l) in the tests and the ordinate axis that of the impact force (i) in arbitrary measuring systems of the

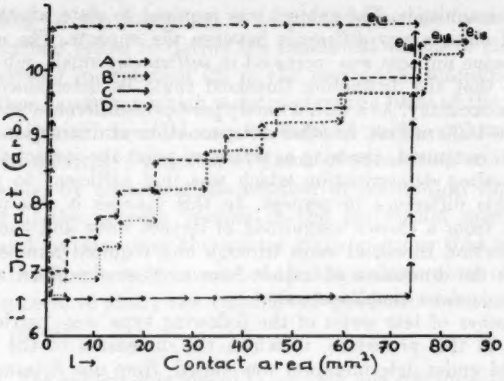

Fig. 2. The equivalence of various types of transpositions in the component structure with intensity and surface dimensions (i, l) of the haptic perceptual manifold. (ε = unit vector defined by the distinction threshold).

stimulus (contact surface area: mm², impact force: c. g. s. units). The vectors entered as arrows in the coordinate system show the distinction thresholds as expressed by the tests subject (unit vectors $\vec{e_l}$ and $\vec{e_i}$); the vectors obtained in the A series have been shown as dash lines, those of the B series as dot lines, of the C series as solid lines and those of the D series as dot-and-dash lines. It can be seen from the figure that proceeding from a given percept (represented in the stimulus sphere by the abscissa value = 1 mm² and the ordinate = 656.8 c. g. s. units) by steps equalling the distinction thresholds, nine steps each in the direction of both dimensions give perceptional values which are very close to each other in the different series. The ultimate results, as represented by the end points of the respective vectors $\vec{e}_{l\,18}$ or $\vec{e}_{i\,18}$, form a scattering pattern, which has projections on the i and l axes not exceeding the distinction threshold at that particular point in the coordinate system. The result is therefore independent of the type of test series employed.

Fig. 3 shows the typical result of a test where the subject performed, in one session, one after the other, one C series and four E series. Abscissae and ordinates are the same as in Fig. 2, as is also the initial stimulus at the beginning of the test (both in the C

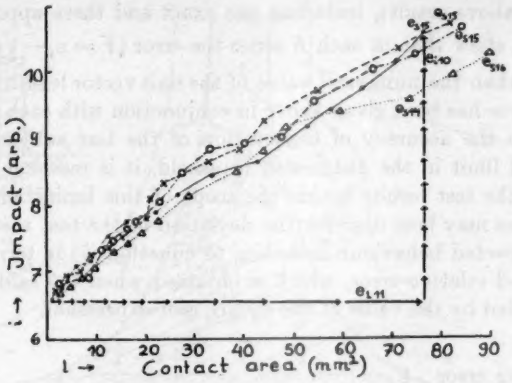

Fig. 3. Transpositions in the component structure with intensity and surface dimensions (i, l) of the haptic perceptual manifold; separately in time in the direction of the i and l coordinates (C series, vectors shown with solid lines) and simultaneously in the i and l dimensions (E series, vectors shown with interrupted lines). e = unit vector defined by the distinction threshold.

and E series). In the C series, in which the progress was 11 steps in the direction of the l dimension (number of $\vec{e_l}$ unit vectors $n_l = 11$) and 10 steps in the dimension i (number of $\vec{e_i}$ unit vectors $n_i = 10$), the ultimate stimulus had the quantity: impulse = 1,070 c. g. s. units, contact area = 75 mm². In the E series, progressing from the same initial stimulus simultaneously in both dimensions, this ultimate value of the stimulus was obtained after 14 to 15 threshold steps (number of $\vec{e_s}$ unit vectors $n_s = 14...$ ....15).

This result can be related to the problem of our investigation as expressed in equation (7) $n_s = \sqrt{n_i^2 + n_l^2}$ by substituting in this equation the empirical values of n_s, n_i and n_l from the tests. The results shown in Fig. 3 will then be represented in the following form:

$n_s = \sqrt{n_i^2 + n_l^2}$	(Question)	Error (V)
$15.0 \approx 14.9$	(solid line)	+ 0.1
$14.0 \approx 14.0$	(dot-dash)	0.0
$14.0 \approx 14.4$	(dash line)	— 0.4
$14.0 \approx 14.4$	(dot line)	— 0.4

The above results, including one exact and three approximate results, show that in each *E* series the error ($V = n_s - \sqrt{n_i^2 + n_l^2}$) is less than the numerical value of the unit vector length $|\vec{e_s}| = 1$. The error has been given above in conjunction with each result.

Since the accuracy of observation of the test subject has its natural limit in the distinction threshold, it is reasonable to describe the test results within the scope of this limitation. In this sense we may best describe the deviation of the test results from the expected behaviour according to equation (7) in terms of the so-called relative error, which is obtained when the said error V is divided by the value of the square root expression:

$$\text{Relative error} \quad V_r = \frac{V}{\sqrt{n_i^2 + n_l^2}} = \frac{n_s}{\sqrt{n_i^2 + n_l^2}} - 1$$

or, if $\sqrt{n_i^2 + n_l^2} = N_s$ (= value from the test result),

$$\text{Relative error} \quad V_r = \frac{n_s}{N_s} - 1.$$

Fig. 4 shows a graphical synopsis of the results from all combined *C* and *E* series tests in a representation chosen on the basis of the preceding considerations. The abscissae are values of the relative error (V_r) and the ordinates correspond to the value N_s (the square root expression) as determined in the *C* series. Each plot in the figure thus gives the results of the distinction threshold determination in the subject's *E* series test, while the abscissa gives the relative error of this result as referred to the *C* series result corresponding to its ordinate. The solid lines in the figure enclose all such plots (shaded area) which represent a result whose relative error does not exceed the distinction threshold in magnitude. The dotted lines similarly indicate the limit corresponding to a relative error equalling twice the distinction threshold.

It can be seen from Fig. 4 that the major part of the results fall into the shaded area, *i. e.* their relative error remains within the limits of the single distinction threshold. The insert in the upper left corner of the figure shows the numerical distribution of the results with regard to the magnitude of their errors. Here, the abscissae show the error in terms of multiples of the distinction threshold and the ordinates the number of individual test results. It is seen that the results are strongly concentrated in the interval between the abscissae of + 0.5 and — 0.5.

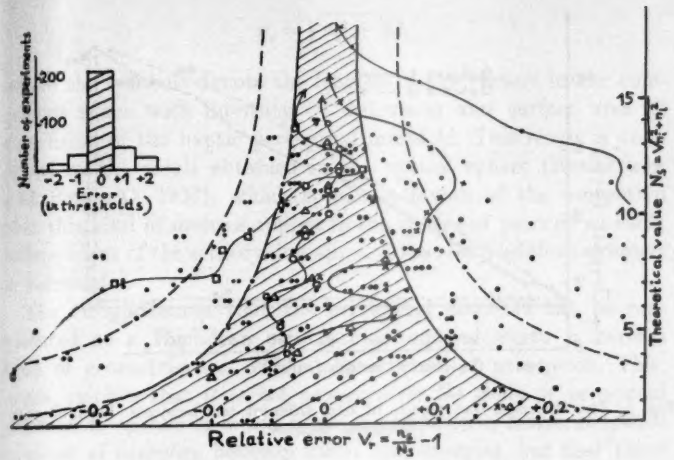

Fig. 4. Comparison between transpositions in the component structure having intensity and surface area dimensions (i, l), occurring separately in time in the i and l dimensions and simultaneously in both dimensions. Abscissae: Difference of the number n_s of distinction thresholds (unit vectors) required in simultaneous transposition, from that N_s consistent with the number of required thresholds at transposition separately in time (n_i and n_l respectively), expressed in terms of relative error $V_r = \frac{n_s}{N_s} - 1$. Ordinates: $N_s = \sqrt{n_i^2 + n_l^2}$, theoretical value given by transpositions separately in time. The insert in the left upper corner shows the numerical distribution of the results in terms of multiples of the distinction threshold (shaded area, error < 1 distinction threshold).

The results of the particular test series described by Fig. 3 have been identified in Fig. 4 by particular symbols (rings, triangles, squares and crosses). The results from the individual *E* series of some particular subjects have also been specially indicated by connecting them with a thin, solid line. This shows that, even though the relative error of one series may be quite considerable, it generally tends to decrease with increasing number of threshold steps involved in the series. Illustrative of this is one particular succession of series (on the right) which shows relative errors liberally in excess of twice the distinction threshold at values $N_s = 1.....15$ but displays a distinct tendency towards zero error at values above this.

Fig. 5 shows the results of two subjects (*L.* and *H.*), the error of both being less than the distinction threshold and thus "ac-

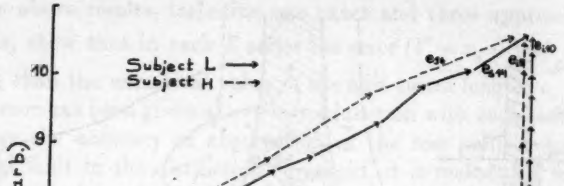

Fig. 5. Two different test results of two different test subjects, both obeying quadratic metrics.

curate" in this sense. This exact result has been achieved in spite of the fact that the two subjects have required a greatly different number of threshold steps in order to proceed exactly the same distance in the coordinate system (the coordinates of the start and end points of the *C* and *E* series being the same in both cases). The distance was traversed, in the *E* series, in 14 steps by subject *L.* and 6 steps by subject *H.*

Conclusions and discussion. The test results presented above support the hypothesis presented in the paragraph relating to the problem, concerning the Euclidean structure of the component continuum with intensity and surface area dimensions of the haptic sphere and the quadratic metrics applying there. This result shows that *it is possible to treat the perceptual ("subjective") manifold as a vector space* (REENPÄÄ 1952, 1953), the natural system of measuring units of which will be determined in accordance with the unit vectors manifesting themselves in the distinction thresholds. The application of numerical values to the perceived quantities has been shown in equations (3), (4), (5) and (6) in the paragraph relating to the problem. Within the limits of accuracy of the distinction threshold, the test results described by means of this application obeyed (cf. Fig. 4) the equation corresponding to the existence of quadratic metrics:

$$n_s = \sqrt{n_i^2 + n_l^2},$$

where the symbols denote the lengths of the vectors in the component space with intensity (i) dimension and surface area (l) dimension of the haptic perceptual manifold. This result is analogous to the result obtained in the optical sphere (BERGSTRÖM and REENPÄÄ 1957), which speaks in favour of the contention that this kind of metrics applies in the sphere of percept as such, independent of the sensory domain, *i. e.* that *the Euclidean structure is intermodal.*

The circumstances that the perceptual manifold can be represented as a Euclidean structure is equivalent to a certain kind of geometrisation of the phenomenon of perception. This, again, implies that *it is not necessary for the study of perceptual processes to operate by means of the investigation of temporal-spatial relations of causality* between forces and energies, but that these processes rather obey the structures given as such in the percept (BERGSTRÖM 1957). This aspect of the problematics of sensory physiology is identical with that at which one has already arrived in physics. An indication of this "structure-physiological" mode of approach is the explanation given to the "summation phenomenon", already presented in connection with an investigation performed in the optical sphere (ref. above): *The summation of the effects of stimulus area and stimulus intensity in the stimulus is "caused" by the Euclidean structure of the percept,* which is a structural-physiological fact existing as such.

The test results obtained in this instance also require an adjustment of the concept of observational accuracy. A limit is naturally set to the "accuracy" of the threshold determination by the length of the "unit vector" determining the distinction threshold. Even though different values are obtained for the length of this unit vector in the stimulus sphere (in the physical, measuring system of the stimulus) for different points in the sphere or for different test subjects (cf. Fig. 5), their length in the natural measuring system of the perception always equals 1. On examination of the test results shown in Fig. 5, where the length of the unit vector of series *E* as expressed in arbitrary units of the stimulus was more than twice as much with subject *H.* as with subject *L.*, it can still be noted that the results of both subjects obey the Euclidean, quadratic metrics without any error exceeding the limit of the distinction threshold. In this latter sense subject *H.*,

who is much "less accurate" than subject *L.* when criticised in the stimulus sphere, shows as good an accuracy as subject *L.* In consideration of such results it is necessary to distinguish between the accuracy of observation which bears relation to the metrics of the experiential structure of a given sensory sphere, and the accuracy of observation which is independent of these metrics but bears relation to the quantities in the stimulus sphere. This latter form is the one usually called accuracy in observation. It is peculiar, however, that this conventional *accuracy of observation, i. e. the ability to distinguish between two stimulus values, seems to be independent of the "accuracy" of obedience to the Euclidean perceptual structure.* This result can be understood since the structural properties of the percept are obviously such fundamental, "deep" functions of observation that they are independent of the more "secondary" distinguishing ability, which is also susceptible to external influence (fatigue, structure and state of the sensory organ etc.). Thus, the accuracy of the distinguishing ability may also vary from one modal sphere to another, whereas the fundamental structure and metrics of the perceptual manifold obviously are intermodal.

The results of investigation presented above speak strongly in favour of the idea that in the act of observation the perceptual occurrence, the percept itself as such, constitutes the structural basis from which the human treatment describing the stimulating "milieu" takes shape. It is difficult in any other sense to understand the suitability of the geometrical disciplines for the description of perceptual functions. In this sense, the tests performed in this connection can be thought to support the Kantian contention of human formation of concepts on the basis of perceptual structures.

Summary.

It is demonstrated experimentally that quadratic metrics apply in the component structures with intensity and surface area dimensions of the haptic type of perception.

Our thanks are due to Mr Olli Tammi, Doct. phil., for his kind assistance in formulating the mathematical expressions involved in this paper.

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Über den Zusammenhang der von voluntärer Muskelaktion erbrachten Bewegungsgrösse und der elektrischen Aktivität des Muskels.

Von

R. M. BERGSTRÖM.

Eingegangen am 9. Juni 1958.

In einer früheren Untersuchung (BERGSTRÖM 1957) liess sich bei leichten, voluntären Aktionen des m. interosseus dors. I eine lineare Abhängigkeit zwischen Anzahl der im Muskel registrierten motorischen EMG-Impulse (Periodenzahl) und dem vom Muskel erzeugten mechanischen Kraftimpuls (als Bewegungsgrösse gemessen) nachweisen. Andererseits hat LIPPOLD (1952) festgestellt, dass es bei isometrischer, dem Willen unterstellter Kontraktion (m. gastrocnemius-soleus) eine lineare Beziehung zwischen der vom Muskel entwickelten physikalischen Kraft und der elektrischen Aktivität des Muskels, d. h. dem integrierten Elektromyogramm besteht. Die lineare Abhängingkeitsbeziehung bleibt bei der Verkürzung des Muskels erhalten, wenn die Verkürzung mit konstanter Geschwindigkeit stattfindet (BIGLAND und LIPPOLD 1954). In den genannten Untersuchungen erfolgte das Registrieren der Muskelaktionsströme mit Hilfe von Flächenelektroden.

Die integrierte, elektrische Aktivität des Muskels kann man, wie LIPPOLD annimmt, als Ausdruck der Anzahl der motorischen Einheiten und ihrer Tätigkeitsdichte, der Frequenz der Aktionspotentialvariation ansehen. Somit scheint es uns angebracht zu untersuchen, welcher Zusammenhang es zwischen dem integrierten EMG und der vom Muskel bei einer voluntären, nichtisometrischen Aktion ermittelten Bewegungsgrösse herrscht.

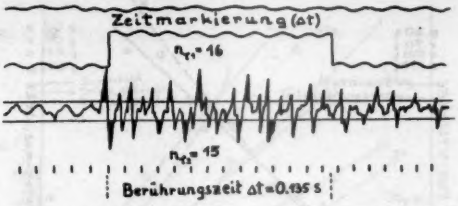

Abb. 1. Berechnung der Anzahl der motorischen Impulse (n_T, schwarze Zacken) im Elektromyogramm, Ausschaltung der Störungszone.

Methodik.

Die Versuche wurden am Abduktionsmuskel des Zeigefingers ausgeführt, in dem die Versuchspersonen die Aufgabe gestellt wurde, mit dem Finger Aktionen auszuführen, welche ein Pendel in Bewegung versetzten (zur genaueren Methodik, siehe BERGSTRÖM 1958). An der Pendelstange, deren eigene Masse auf die Stossstelle reduziert einer Masse von 20 g entsprach, wurden bei den Versuchen Massen von 0, 50, 100 und 150 g angebracht. Die erzeugten Schwingungsamplituden variierten zwischen 5 und 20°. Aus den Ergebnissen wurden in jeder Gruppe mit verschiedener Schwingmasse diejenigen ausgesondert, in denen sich eine Schwingungsamplitude von 5, 10, 13 bzw. 20° (innerhalb einer zugelassenen Streuung von $\pm$ 0.5°) ergeben hatte. Derart wurden für jede Masse und jede erwähnte Schwingungsweite bei allen fünf benutzten Versuchspersonen je 6 brauchbare EMG-Aufzeichnungen erhalten. Beim Behandeln der Ergebnisse als Gesamtheit lagen also für jede Masse und Schwingungsweite 30 Bestimmungen der Bewegungsgrösse und integrierten EMG-Aktivität vor. Die Aufzeichnung der Aktionsströme des m. interosseus dors. I erfolgte mit Hilfe von Flächenelektroden, Verstärker und Kathodenstrahloszillograph (Filmgeschwindigkeit 50 cm/sec) in üblicher Weise (siehe BERGSTRÖM 1958). Für gleichortige Anbringung der Elektroden sowie für gleicher Verstärkungsgrad bei den verschiedenen Versuchen wurde gesorgt. Die Berechnung der integrierten, elektrischen Aktivität aus dem EMG geschah planimetrisch. Zugleich wurde aus dem EMG die Zahl der motorischen Impulse nach der Arbeit von JALAVISTO *et al.* (1938) berechnet (Abb. 1).

Ergebnisse. In der graphischen Darstellung der Ergebnisse in Abb. 2 geben die Abszissenwerte die an die Pendelmasse erteilten Bewegungsgrössen dar, während als Ordinatenwerte links die entsprechenden Impulszahlen und rechts die Werte der integrierten Aktivität des EMG eingetragen sind. Jeder dargestellte Punkt vertritt das Mittel aus 30 Beobachtungen. Die mittlere Abweichung belief sich auf $\pm$ 0.55 bis $\pm$ 1.31 Impulzsahl-Einheiten. Man sieht, dass bei den verschiedenen Massen die Impulszahl (n_T) geradlinig

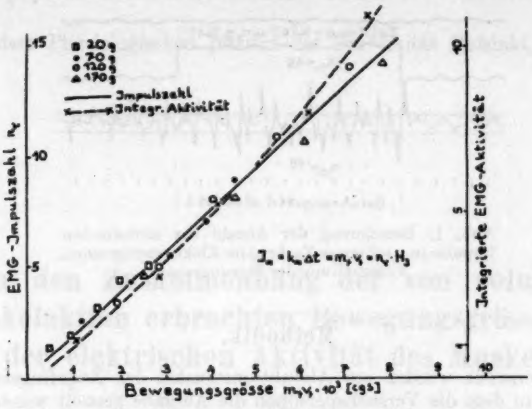

Abb. 2. Die bei einer Zeigefingerabduktion erteilte Bewegungsgrösse und integrierte, elektrische Elektromyogramm-Aktivität bzw. Zahl der Elektromyogramm-Impulse.

mit zunehmender Bewegungsgrösse ($m_f \cdot v_f$) wächst (ausgezogene Linie), wogegen die integrierte EMG-Aktivität (gestrichelte Linie) eine nach oben konkave Kurve zu ergeben scheint. In Abb. 3 ist die integrierte EMG-Aktivität (gestrichelte Linien), bzw. die Impulszahl (ausgezogene Linien) in Abhängigkeit von der Anfangsgeschwindigkeit der verschiedenen Pendelmassen dargestellt worden. Auch hier ergeben sich für die Impulszahl recht gut geradlinige Darstellungen, wogegen die Kurven der integrierten Aktivität auch hier gebogen sind. Die punktierten Linien geben die der verschiedenen Anfangsgeschwindigkeiten zugehörigen Bewegungsgrössen an (Ordinate $m_f \cdot v_f$). Die beinahe systematische Abweichung der Impulszahlwerte bei hohen Geschwindigkeiten gegen die Abszisse hin, ist wohl auf den Umstand zurückzuführen, dass die Überdeckung der EMG-Impulse bei hohen Frequenzen die Zahl der Impulse vermindert.

Besprechung. Gemäss einer früheren Untersuchung liess sich aussagen, dass bei einer voluntären Muskelaktion der vom Muskel hervorgebrachte mechanische Kraftimpuls (durch die von der Aktion bewirkte Bewegungsgrösse gemessen) der totalen Anzahl der während der Muskelaktion registrierten Impulse im Elektromyogramm verhältnisgleich ist (siehe auch Abb. 2). Dieses Resultat liess sich folgendermassen ausdrücken:

$$I_m = k_m \cdot \Delta t = m_f \cdot v_f = n_\tau \cdot H_I,$$

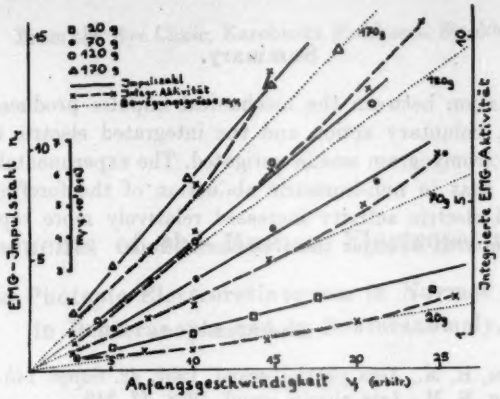

Abb. 3. Die bei der Zeigefingerabduktion den verschiedenen Pendelmassen erteilte Anfangsgeschwindigkeit und integrierte EMG-Aktivität bzw. Zahl der EMG-Impulse.

wo I_m der vom Muskel bei der Aktion hervorgebrachte Kraftimpuls, k_m die vom Muskel entwickelte Kraft, $\triangle t$ die Wirkungsdauer dieser Kraft, n_τ die Gesamtanzahl der EMG-Impulse und H_I eine Konstante mit der Diemension (g.cm.s^{-1}) ist, (BERGSTRÖM 1958).

Da sich die integrierte, elektrische Aktivität des Muskels auf die Gesamtanzahl der Aktionspotentiale im Muskel zurückführen lässt, wäre, gemäss obiger Gleichung eine lineare Beziehung zwischen integrierter EMG und Bewegungsgrösse zu erwarten. Die dargelegten Versuchsergebnisse zeigen jedoch, dass das nicht der Fall ist. Es besteht somit die Möglichkeit, dass hier entweder ein Faktor mitspielt, der von den nicht-isometrischen Versuchsbedingungen herrührt, oder wird die verschiedenartige Korrelation der integrierten Aktivität und der Impulzsahl des EMG von der Überdeckung der Muskelaktionspotentiale bei der Registrierung bewirkt.

Zusammenfassung.

Der Zusammenhang zwischen dem bei voluntärer Aktion eines Muskels erbrachten Kraftimpuls und der integrierten, elektrischen Aktivität im Elektromyogramm wurde untersucht. Die Versuchsergebnisse zeigen, dass bei der nicht-isometrischen Zeigefingerabduktion die integrierte, elektrische EMG-Aktivität bei stärkeren Aktionen relativ schneller zuwächst als bei schwächeren.

Summary.

The relation between the mechanical impulse produced by a muscle in voluntary action and the integrated electric activity in the electromyogram was investigated. The experimental results indicated that in non-isometric abduction of the forefinger the integrated electric activity increased relatively more rapidly in connection with stronger than weaker actions.

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Components of the Human Electroretinogram.

The Photopic Electroretinogram in Normal Eyes, in Deuteranopia and in Deuteranomaly.

By

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In a previous paper, a preliminary report was given of the photopic components of the human electroretinogram (ERG), as well as of a special study of the deviations in protanopia (HECK and RENDAHL 1957). A survey was also presented of earlier electroretinographic investigations in this field. Since then, additional studies have been published on the human ERG on stimulation with monochromatic light, with particular interest focused on the differentiation between scotopic and photopic components in recordings from normal eyes (BEST and BOHNEN 1957, BIERSDORF and ARMINGTON 1957, BORNSCHEIN, GOODMAN and GUNKEL 1957, GOODMAN and BORNSCHEIN 1957, RONCHI and MORELAND 1957, RONCHI and STROCCHI 1957). In a recent publication, BORNSCHEIN and GOODMAN (1957) recorded a photopic ERG with two negative and three positive humps, but they have not yet reported any detailed analysis of these components.

An account is given in the present paper of further studies on the photopic ERG in man. A description of the slightly modified apparatus is followed by a report of investigations of the photopic ERG in subjects with normal eyes, both on varying light adaptation and in the course of dark adaptation. The results are then reported of recordings in subjects with deuteranopia and deuteranomaly. Finally, these results are discussed against the background of earlier observations in various types of colour blindness.

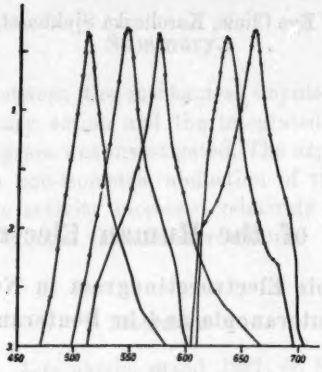

Fig. 1. Transmission curves for the colour filters. *Abscissa:* wave length (mμ). *Ordinate:* transmission (— log).

Methods.

The methods were essentially the same as those used earlier (Heck and Rendahl 1957), but the ERG could be recorded during adaptation to monochromatic light, instead of after previous adaptation to the coloured light.

The ERG was recorded with a contact-glass electrode according to Karpe (1945), but with a matt contact glass. The potentials were amplified by a condensor-coupled amplifier, in most cases with a time constant of 70 msec (possible range of variation: 7,000, 700, 70 and 7 msec). Each curve was recorded on a stationary film with synchronization over a cathode-ray oscillograph of Cossor type. Most recordings imply photography of about 10 superimposed ERGs.

A Philips Tungsten Ribbon lamp 6 V/16 amp., with a colour temperature of about 2,800° K, was used as a stimulus light and for simultaneous light adaptation. An electromagnetically regulated shutter system gave a constant flicker with 1 stimulus per second (same period of light and dark). The intensity of this "white" light was 200—300 lux, measured at the site of the eye, behind the contact glass.

Recordings were also made during adaptation to coloured light, the stimulus light being the same as before. As adaptation light was used another Philips Tungsten Ribbon lamp (6 V/16 amp., 2,800° K), the light passing interference filters with narrow spectral bands and maximal transmission at 518, 545, 572, 636 or 657 mμ, respectively (Fig. 1).

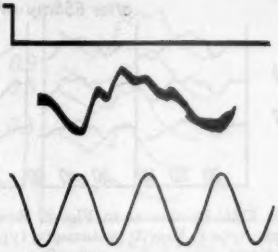

Fig. 2. Photopic ERG in man on stimulation with flickering light, intensity 250 lux, frequency 1/sec (same period of light and dark). Uppermost record: light marking. Middle record: ERG. Bottom record: time marking 50/sec (HECK and RENDAHL, Acta physiol. scand. 1957).

The stimulus light and the adaptation light were then focused on the contactglass by two prisms.

Although the maximal energy was not the same in different parts of the spectrum, it was possible with neutral filters to obtain an approximately equal energy spectrum.

This lamp was also used for "light adaptation" (in addition to the light from the flickering stimulus). The colour temperature was, as already stated, 2,800° K, and the light intensity about 600 lux at the site of the eye. In some experiments the lamp was used together with a pale blue photometric filter (Bausch and Lomb) for converting light from the tungsten filament lamp to approximate Mean Noon Sunlight, this kind of adaptation being denoted as "daylight adaptation", the intensity being about 200 lux.

Experimental Subjects.

All the experimental subjects had clinically "healthy" eyes with full visual acuity. Slight myopia (at most — 2 D) was accepted. The colour sense was tested with pseudo-isochromatic charts and with Nagel's anomaloscope.

Normal eyes: 6 subjects (5 men, 1 woman).
Deuteranopia: 6 subjects (all men).
Deuteranomaly: 4 subjects (all men) and one (a man) with extreme deuteranomaly.

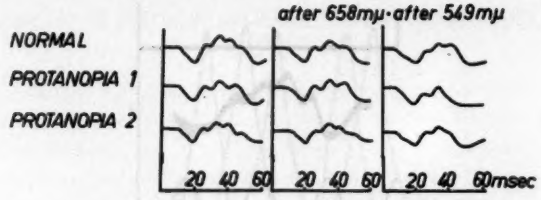

Fig. 3. Photopic ERG in man as in Fig. 2. Row 1: normal eyes. Row 2: protanopia, type I. Row 3: protanopia, type II. — Stimulus light white in every case, before and after pre-adaptation to coloured light as indicated (HECK and RENDAHL, Acta physiol. scand. 1957).

Results.

In the previous investigation, we were able to record a photopic ERG with two negative and four positive humps (Fig. 2). In normal eyes, the third positive wave was temporarily suppressed by pre-adaptation to red light, and the fourth wave by pre-adaptation to green light (Fig. 3). In protanopia, the third component was either lacking, or was present but was uninfluenced by adaptation to red light (Fig. 3).

On examination of one deuteranope, an ordinary photopic ERG with all the components was obtained, but the fourth wave was not suppressed by pre-adaptation to green light.

1. Normal Eyes.

Six subjects with normal eyes were studied with the slightly modified technique. The stimulus used for adaptation to red light had a maximal intensity at 657 or 636 mμ, whereas that of the green light lay at 545 or 518 mμ (see Fig. 1). The red adaptation light was weaker than that used in the experiments reported earlier, and the influence of red adaptation was therefore less pronounced. The results were nevertheless essentially the same as the earlier ones (HECK and RENDAHL 1957).

Influence of different light adaptation. The eye was adapted both to flickering light and, in some experiments, simultaneously to "white light" of varying intensity (*cf.* Methods). With the strongest available intensity of flickering light only, an ordinary photopic

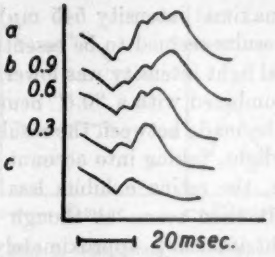

Fig. 4. Photopic ERG on increasing light adaptation. a) Ordinary photopic ERG as in Fig. 2. b) Flickering light stimulus as before, with concurrent, continuous light adaptation (see text). The numerals denote the transmission of the neutral filters (— log). For details: see text. c) Strong light adaptation. The photopic ERG is reduced to *one* negative and *one* positive wave.

ERG was obtained. With a decrease in intensity of the stimulus light, the various components became confluent and the potentials diminished, but the duration of the ERG was unaltered.

With a constant maximal intensity of the light stimulus, the light adaptation of the eye was then increased, the intensity of the adaptation light being varied by means of neutral filters (strength given as density). Use of "daylight adaptation" and a "1.2" neutral filter produced a slight decrease in the last two components, and shorter peak latencies were obtained (Fig. 4). With a "0.9" filter, the third hump decreased still more, and the fourth hump appeared earlier than normally (the tracing resembled that in adaptation to red light). On further light adaptation ("0.6" filter) the curve consisted of the two negative components and the first three positive humps (same tracing as on adaptation to green light). With a "0.3" neutral filter, only the first two positive humps appeared, as in maximal "daylight adaptation" (without neutral filter). If the daylight filter as well is removed, the light intensity is increased about three times; the photopic ERG then consisted of only one negative wave (a_1) and one positive hump.

The apparatus used did not permit any further increase in either light adaptation or intensity of the flickering light.

If a green light (maximal intensity 545 mμ) was used instead for adaptation, the results seemed to be essentially the same, except that the maximal light intensity was lower, and corresponded to a daylight filter combined with a "0.6" neutral filter.

A comparison can be made between the results with adaptation to green and to red light, taking into account that, although the red light is stronger, the retina exhibits less sensitivity in this wavelength region. It then seems as though the curves varied parallel with the light intensity, approximately as in adaptation to "white" light.

When light with a maximal intensity at 572, 545 or 518 mμ was used for green adaptation, about the same light energy was required, irrespective of the wavelength, to produce bare suppression of the fourth positive hump (one case). This implies that at 545 mμ (three cases) the green light was weakened by addition of a "0.6" neutral filter (two cases) or a "0.9" filter (one case).

The blue adaptation light was considerably weaker than the green. It did not influence the components of the photopic ERG.

To sum up, it can be stated that with increasing light adaptation, but unaltered light stimulus, typical changes appeared in the photopic ERG, the various components being suppressed successively, starting with those having a longer peak latency. On adaptation to monochromatic light, the last two components were affected; this influence also seems to be explainable by the varying intensity of the light stimulus.

Monochromatic light was, however, chiefly used for adaptation in examination of colour-blind subjects, to increase the possibility of disclosing eventual differences from the results in normal subjects. In a few experimental series, the variations with different degrees of light adaptation were recorded concurrently.

Influence of incipient dark adaptation. In one experimental series, the effect was studied of incipient dark adaptation on the photopic ERG of a subject with normal eyes. After strong light adaptation (about 1,000 lux at the site of the eye) the ERG was recorded both on stimulation with a slow flicker and with single flashes.

The flicker ERG (slow flicker) was recorded during the first 10 minutes of dark adaptation every minute (10 sec each time) and thereafter every five minutes (about 30 sec each time). The single-flash ERG was recorded at an intensity of light stimulus of 10 lux (at the site of the eye) and a flash duration of 1/25 sec. These single flashes

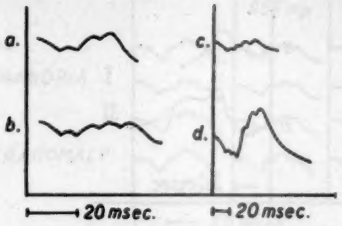

Fig. 5. Variations in the photopic ERG during dark adaptation. (Light stimulus: flickering light, as in Fig. 2.) a) Immediately after light adaptation: 2 neg. and 3 pos. components. b) and c) After 1 min.: ordinary photopic ERG with 2 neg. and 4 pos. components. d) After 25 min.: photopic and scotopic components (3 neg. and 5 pos. waves recorded).

presumably did not affect the adaptation to darkness (KARPE and TANSLEY 1948), whereas the flickering light must certainly have retarded the course of adaptation.

Immediately after light adaptation, a flicker ERG was obtained in which the fourth positive wave was lacking. In other respects, the curve had an ordinary appearance (Fig. 5).

After one minute (all times apply from the moment when the adaptation light was turned off), an ordinary photopic ERG with all the components was recorded (Fig. 5).

After five minutes, the first single-flash ERG was recorded (Fig. 6). It started with a distinct *a*-wave, which in size and latency corresponded to a_1 in the photopic ERG recorded earlier. A negative wave followed, and then two positive, not distinctly separate waves and, finally, a strikingly conspicuous *b*-wave, which corresponded in size and latency to the fourth hump of the photopic ERG. This curve recorded on single-flash electroretinography has been interpreted as a photopic ERG in which the components are less distinct, chiefly on account of the weaker light stimulus.

With single flashes, an ERG with a probable third negative wave was obtained after 15 minutes, following the first flash; after 23 minutes, this type of ERG was recorded after every flash (Fig. 6). From the point of view of time, this third negative wave (a_3) lay between the second and third positive waves of the photopic

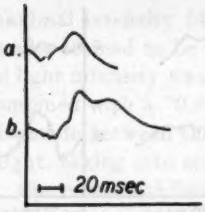

Fig. 6. Same as in Fig. 5, but different light stimulus: single flashes with an intensity of about 10 lux. a) After 5 min.: probably photopic ERG with indistinctly marked components. b) After 23 min.: photopic and scotopic components (3 neg. and 5 pos. waves).

ERG. Simultaneously with a_3, a distinct *b*-wave appeared with a peak latency of 80 msec after the positive waves observed earlier (which were no longer so distinctly demarcated). This *b*-wave was interpreted as being identical with the scotopic *b*-wave.

The last recordings during this experiment were made after 25 minutes, using a flickering light. With the first light stimulus (thus, when the eye was dark-adapted) an ERG was obtained with three negative waves, of which a_1 and a_2 coincided in time with the same components of the photopic ERG, whereas a_3 reached its deepest point 37 msec after the beginning of the light stimulus (Fig. 5). Moreover, in view of the peak latencies, the four positive humps of the photopic ERG seemed to be present, followed by the scotopic *b*-potential.

Thus, in the course of dark adaptation, the ERG recorded in a subject with normal eyes had three negative and five positive humps. This ERG therefore appears to contain both the photopic and the scotopic components of the ERG.

2. Deuteranopia.

The photopic ERG was recorded in six deuteranopes. On stimulation with a slow flickering light, an ordinary curve with two negative and four positive humps was obtained. On adaptation to red light (657 or 636 mμ) the third positive component was decreased

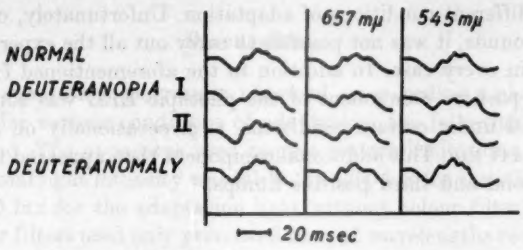

Fig. 7. Photopic ERG in man in deuteranopia and deuteranomaly (*cf.* Fig. 3). Row 1: normal eyes. Row 2: deuteranopia, type I. Row 3: deuteranopia, type II. Row 4: deuteranomaly. On adaptation to green light, a distinct remains of the fourth positive wave is visible. — Stimulus light white in every case, before and during adaptation to coloured light as indicated.

or suppressed, whereas the fourth hump exhibited somewhat shorter peak latency than earlier. The result was the same as in normal eyes.

On adaptation to green light (545 or 518 mμ), two types of deuteranopia could be distinguished.

In *type I* (two cases), four positive components were present on adaptation to green light as well; the fourth component even seemed to be slightly increased (Fig. 7). One of these cases is that reported earlier by HECK and RENDAHL (1957).

In *type II*, the fourth positive hump was suppressed on adaptation to green light, as in normal eyes. In two of these deuteranopes, a small remainder of the fourth wave nevertheless seemed to persist directly after the third positive wave (Fig. 7).

In order barely to suppress the fourth component, the adaptation light had to be weakened by addition of neutral filter "1.2" (two cases).

Adaptation to "white light" (two cases) or to "daylight" (one case) produced no definite deviations from the results in normal eyes.

In *extreme deuteranomaly*, the results were practically the same as in type II deuteranopia. On adaptation to green light, a small remains of the fourth component was visible; a "0.9" neutral filter was required for its exact suppression.

Thus, to sum up, a study was made of the way in which the components of the photopic ERG in deuteranopes are altered

under different conditions of adaptation. Unfortunately, on practical grounds, it was not possible to carry out all the experimental stages in every case. In addition to the aforementioned findings, a fifth positive component of the photopic ERG was sometimes observed under certain conditions, *e. g.* occasionally on adaptation to 441 mμ. This additional component then appeared between the second and third positive humps.

3. Deuteranomaly.

Four subjects with deuteranomaly were examined. With the usual slow flicker, they exhibited an ordinary photopic ERG with all the components. On adaptation to red light (636 mμ) the third component decreased, as far as could be judged in the same way as in normal eyes. On adaptation to green light (572, 545 or 518 mμ) the fourth positive component was affected in three cases in a way differing from that in normal eyes.

In these three experimental subjects, a small remains of the fourth component was invariably present immediately after the third hump (Fig. 7). To produce bare suppression of a normal fourth positive wave, the monochromatic adaptation light could not be weakened more than by addition of a "0.3" neutral filter (at 545 mμ), and the same light energy was required to suppress the hump at 572 or 518 mμ.

In one subject, the results did not differ from those in examination of normal eyes. The adaptation light could be weakened with a "0.9" neutral filter to produce bare suppression of the fourth component.

In this subject, as well as in one of the aforementioned, recordings were also made with "light adaptation" and "daylight adaptation". The results did not differ with certainty from those in normal eyes.

To sum up, the following statement can be made. It seems as though in some cases of deuteranomaly, a greater intensity of light is required — at any rate if green light is used for adaptation — to suppress the fourth positive component of the photopic ERG.

Discussion.

When evaulating the results obtained on recording a photopic ERG under various conditions of adaptation, the following must be stressed. The apparatus used implies certain limitations, since the maximal light intensity was fairly low (250 lux for the stimulus light, 600 lux for the adaptation light without colour filter), and the colour filters used only permitted tests at wavelengths relatively far apart (at steps of 20—30 mμ). Moreover, the energy data must be regarded as somewhat approximative, since they took place at steps of 0.3 logarithm units.

In examination of normal eyes under conditions of light adaptation, it was found that the intensity of the adaptation light was probably of greater importance than the colour of the stimulus light for suppression of the various components of the photopic ERG (Fig. 4). Monochromatic light was, however, chiefly used for examination of colour-blind subjects, and it seems as though differences between normal and colour-blind eyes, at any rate, appear more distinctly on adaptation to coloured light.

When recordings were made from normal eyes in the course of dark adaptation, a third negative wave and a late positive wave seemed to appear (Fig. 5 and 6). This clearly supports the inference that the two negative and four positive humps observed are purely photopic components. If they are to be sharply demarcated, a relatively strong light stimulus and a large illuminated field are required. These were ensured in the present investigation by using a matt contact glass, which spread the light. If the intensity of the light stimulus is decreased, or a clear contact glass is used, longer latencies are obtained for the components and, furthermore, they can no longer be clearly distinguished from each other.

In deuteranopes, stimulation by flickering light produced an ordinary photopic ERG with all the components. On adaptation to green light, two types of curve were, however, obtained. In type I (two cases) an unchanged ERG appeared, whereas in type II (four cases) the fourth positive hump was suppressed, as in normal eyes (Fig. 7). If the green adaptation light was weakened by addition of neutral filters (two cases) it was found that only $^1/_{16}$ of the maximal intensity was required for exact suppression of the fourth component ("1.2" neutral filter), as compared to $^1/_4$—$^1/_8$ ("0.6"—"0.9") in normal eyes (three cases). In type I,

the fourth wave even seemed to be increased on adaptation to green light. Since the photopic ERG is composed of several negative and positive components, this has been interpreted to indicate that some displacement in the latencies must have occurred, thus permitting one wave to be more conspicuous than normal, whereas it is less likely that any increase has, in fact, taken place in the component in question.

In one subject with extreme deuteranomaly, the results were practically the same as those in type II deuteranopia.

One of the four subjects with deuteranomaly (no gradation of the colour-vision defect in a colorimeter was possible) exhibited completely normal conditions. In the other three, an ordinary photopic ERG was recorded with flickering light only. However, on adaptation to green light (maximal intensity) the fourth positive wave could barely be suppressed, and when the intensity of the adaptation light was reduced to half ("0.3" neutral filter), the fourth hump promptly reappeared. Thus, the results in these subjects closely resembled those in type I deuteranopes.

Briefly, this study of the photopic ERG has shown that, in normal eyes, the components recorded can be successively suppressed by increasing the light adaptation with both "white" and monochromatic light of varying wavelength. It is true that it could not be established whether the intensity or the wavelength was decisive in this respect. It is nevertheless clearly apparent, both from the present investigation of colour-blind subjects and from the earlier one (HECK and RENDAHL 1957), that in such cases the photopic ERG often differs distinctly from that of normal eyes, both in appearance and in the reactions to altered conditions of adaptation. Of the four positive humps, the third is affected in the presence of defects in red vision, so that it is either lacking (type I protanopes), or is uninfluenced by adaptation to red light (type II protanopes).

As far as deuteranopes are concerned, two types were also distinguished. Both exhibited an ordinary photopic ERG with slow flickering light only, whereas adaptation to green light failed to suppress the fourth wave in some of them (type I). In the others (type II) it was suppressed on adaptation to green light, but a lower intensity was possibly required than in normal eyes.

The records of most of the subjects with deuteranomaly seem to correspond fairly closely to those in type II deuteranopia, with a fourth component exhibiting decreased sensitivity to green light.

A comparison can be made between these results in deuteranopia and the investigations published by WILLMER (1949) on the spectral sensitivity of the fovea in persons with colour-vision defects. Good agreement is then found with his view that two types of deuteranopia exist, *i. e.*, one type in which the retina lacks green-sensitive receptors (corresponding to my type I), and another type with normal receptors, in which the impulses from red and green receptors are, however, coupled to the same nerve paths (corresponding to my type II). The deuteranomalous colour-vision defect is best explained by a decreased sensitivity to green, and the electroretinographic studies are in good conformity with this interpretation.

In his interesting studies of the retinal pigment *in vivo*, RUSHTON (1955) reported that in examination of one deuteranope (and one patient with marked deuteranomaly) he had found the macula to be poor in photopic pigment. This seems to be compatible with the electroretinographic tendency to decreased sensitivity to green adaptation light in some deuteranopes.

Summary.

1. A photopic electroretinogram (ERG) with two negative and four positive humps was recorded earlier (HECK and RENDAHL 1957). In the course of dark adaptation, an ERG has now been recorded which appears to contain one additional negative wave and one positive one, evidently the scotopic components.

2. It has been found possible to suppress the various components of the ERG successively by increasing the light adaptation.

3. On examination of six deuteranopes, an ordinary photopic ERG was obtained. On adaptation to green light, the fourth component was unaffected in two cases. A markedly decreased sensitivity to green light was observed in a similar way in three subjects with deuteranomaly (a fourth exhibited the same variations as in subjects with normal colour sense).

4. In the four other deuteranopes, the fourth component was suppressed on adaptation to green light, exactly as in normal eyes; the sensitivity to green light was perhaps increased.

5. On the basis of this and other investigations of colour blindness, the suggestion is put forward that the first type of deuteranopia is due to a lack of "green receptors" in the retina, whereas

the second type is to be ascribed to the impulses from retinal receptors with different colour sensitivity being coupled to the same nerve paths.

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